31.10.

VOL. XVII, PART III Published 11 November 1932

The British Mycological Society

(*Recognosce notum, ignotum inspice*)

TRANSACTIONS

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Price to non-members, Seven Shillings & Sixpence

LONDON
CAMBRIDGE UNIVERSITY PRESS
FETTER LANE, E.C. 4
CHICAGO: THE UNIVERSITY OF CHICAGO PRESS
(AGENTS FOR THE UNITED STATES)
BOMBAY, CALCUTTA, MADRAS: MACMILLAN
TOKYO: MARUZEN COMPANY LTD.

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PRINTED IN GREAT BRITAIN

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BACTERIAL DISEASES OF STONE-FRUIT TREES IN BRITAIN

IV. THE ORGANISM CAUSING BACTERIAL CANKER OF PLUM TREES

By H. WORMALD
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(With Plates VII and VIII)

Bacterial Canker of plum trees has been described in previous articles (1, 3, 5), and the chief cultural characters of the bacterium isolated from the lesions have been outlined (3). No details were given, however, of the inoculation experiments which led to the conclusion that the organism produced the cankers. As the cause of Die-back in plum trees has been a controversial subject for many years* it seems essential that particulars of these experiments should be published, together with an account of the organism in sufficient detail to allow of its ready identification.

As the symptoms accompanying Bacterial Canker have already been described fully (3), it will be sufficient to summarise them here. Diseased trees in plantations bear yellowish foliage in the spring, the leaves being often "rolled"; later the foliage withers or there is early defoliation. During the summer a canker appears on the stem, or, where a branch only is affected, on the branch itself. On nursery trees the symptoms are similar but usually more pronounced, and some trees may make no growth whatever in spring. The bacteria in the cankers die during the summer and the lesions then cease to increase in size; the bacteria are succeeded by various fungi which are probably merely saprophytes living on the tissues already killed by the bacteria. During the summer, however, infection of the leaves occurs, causing bacterial leaf spots, while occasionally lesions also appear on young shoots and fruit.

The organism has been isolated not only from cankers on stems and branches but also from lesions on leaves, young shoots and fruit. During the years 1926 to 1930 more than thirty strains† have been obtained which have proved by inoculation experiments to be pathogenic and to give very similar cultural reactions. Fourteen of these

* The various explanations which have been put forward previously to account for die-back in plum trees have already been discussed (3, 4).

† By "strain" is here meant a pure line obtained by isolation from a single colony; any one strain may or may not be distinguishable from others by slight cultural differences.

have been isolated from stem or branch lesions of trees in plantations, two from stems of maiden trees in nursery rows, eleven from leaf spots, three from lesions on young shoots, and two from fruit spots.

Isolations from the lesions on leaves, shoots and fruit are easily effected by the usual methods. If the spots are young and fresh the bacteria ooze out in great numbers on teasing out the infected tissues in a little water, and isolation plates can be prepared from the suspension.

In preparing isolations from the older tissues of stems and branches it is essential that the attempts be made early in the season, as a rule not later than June. From the middle of May to early June is a suitable period, for by that time the symptoms are usually evident enough for diseased trees to be recognised, and as the cambium has resumed its activities the outlines of the cankers are showing. If the limits of the canker are ill-defined externally they may be determined by cuts made in the bark at different levels. When the limits of a lesion are approximately fixed a strip of bark is cut off which, when examined on its cut surface, should show clearly the demarcation of healthy and necrotic tissues. If a particle of the dark brown tissues of the cortex at this region is teased out in water, masses of bacteria ooze out, and after waiting an hour or so for the rods to become dispersed through the water, aided by stirring occasionally with a platinum wire loop, isolation plates can be prepared in the usual way.

During the summer (when the cankers are more obvious, and when diseased trees, though still alive, become conspicuous by reason of their pale foliage and arrested growth) attempts at isolation from stem lesions usually fail, and this fact doubtless explains why previous workers have failed to obtain pathogenic bacteria from the cankers.

The culture medium used for most of the isolations was nutrient agar containing 5 per cent. saccharose, since it was found that on this medium the organism produces colonies with characteristic radial lines which aid in its identification.

The first isolations from stem lesions were made in April and May, 1926, and during the latter half of June isolations were made from lesions on leaves, shoots and fruit. Preliminary inoculation experiments were carried out during that summer by inserting the organism into leaves, fruits and young shoots, by means of punctures; the results showed that the strains were pathogenic, for dark brown lesions appeared round the inoculated points and not at control punctures.

More critical experiments were carried out in 1926 and in 1927, using the same strains, and those giving the most interesting results are described below. The inoculations on stems, branches and woody twigs were made in October or November, the results showing during the following spring and summer.

Inoculation experiments

Expt. 26/21*. The trees used for this experiment were two-year-old Victoria plum trees, worked low (union just above ground level). Ten were inoculated and these alternated with ten control trees. The organism used in this experiment was isolated from a stem canker. The inoculum was a turbid suspension of the organism made by stirring up in sterile water the growth from a two-day-old culture on nutrient agar + 5 per cent. saccharose. A Λ-shaped cut was made near the middle of the stem, the tongue of bark turned outwards, and a drop of the suspension inserted. The bark was pushed back into place, and the cut tied round with moist, sterile, absorbent cotton-wool and raffia. The control trees were similarly treated but inoculated with sterile water only. The inoculations were made on October 4, 1926.

During the winter no difference could be detected between the inoculated trees and the controls. When the trees were coming into leaf early in April there was still little difference outwardly, except that, on the whole, the inoculated trees were less forward than the controls, but the buds had swollen and were bursting on all of them. On cutting notches into two of the inoculated trees it was seen that the bark was brown for several inches from the point of inoculation. A particle of bark taken from one inoculated tree, at one foot above the inoculated spot, was found to be swarming with bacteria.

At the end of April the control trees were all coming into leaf with leaves up to two and a half inches long. The inoculated trees all showed evidence of infection, but in different degrees. Three appeared almost normal, but their leaves were no longer than one and a half inches and therefore smaller than in the controls, and some leaves were turning brown. On three others the leaves borne terminally on the twigs were about one and a half inches long, the others noticeably smaller. On each of the other four trees one or more buds had developed leaves from one to one and a half inches long but most of the buds had only swollen and had then turned brown.

By July 22nd the cankered areas were showing clearly, extending upwards and downwards from the points of inoculation for eight to twenty inches; the cankers were therefore mostly about two feet long and had completely girdled the stems. Seven of the trees were quite dead above the cankers, two had leaves just beginning to wilt and one was defoliated except for two green leaves. Fungal fructifications were now appearing on the cankers.

* Each experiment is denoted by two numbers, the first indicating the year in which the experiment was carried out, the second being the serial number of the experiment.

On July 9th all were dead above the cankers. One tree was killed to ground level, so that no shoots grew out below the canker (Fig. 3); in two the scion was killed to the base but shoots grew out from the stock; in the rest, a few inches of the scion stem below the canker remained alive and the buds there grew out to form long vigorous shoots.

Expt. 26/22. Sixteen four-year-old Victoria trees were used, in two sets, (*A*) eight worked on Brompton stocks, and (*B*) eight on Brussels. In each set two were inoculated at two places on the stem (one near the middle of the stem, the other two inches higher on the opposite side) and two at one place only. There were corresponding control trees. The organism used for the inoculations was one isolated from a leaf spot.

At the end of April the trees were indistinguishable in general appearance. The infected areas were faintly marked by longitudinal ridging at their edges on four of the inoculated trees. In May the infected areas became more pronounced as the healthy parts of the stem began to increase in thickness. By the middle of July the foliage of the two trees in set (*A*) which were inoculated at two places showed the symptoms typical of trees with Bacterial Canker; the leaves were yellowish and their margins were curled upwards. These two trees became defoliated by the end of September. On the two trees inoculated at one place only, cankers developed which did not girdle the stems; one had a canker nine inches long, half girdling, the other a canker thirty-five inches long, three-quarters girdling along the greater part of its length but nowhere quite girdling, and the tree survived. The wounds on the control trees healed normally without any cankering.

In set (*B*) the results were less striking; a canker arose at each inoculated spot, the largest being seven inches long, and at one of the control wounds a small canker developed.

Expt. 26/28. Inoculations were made on plum branches using three strains isolated from stem cankers. With each strain two branches were inoculated at two places each (twelve inoculations in all, with corresponding controls). Cankers arose at all points of inoculation, and three of them girdled the branches, causing the death of the parts terminal to them.

Expt. 26/29. Branches one-third to three-quarters of an inch thick were inoculated using two strains from leaf spots. Cankers arose at all the inoculated places; two of the branches became girdled and died. All control wounds healed normally.

Expt. 26/31. Woody twigs and one-year-old branches were inoculated using two strains from shoot lesions and two from fruit lesions, one twig and one branch with each strain. Three of the twigs became girdled and were killed before the buds had swollen

so that they made no growth whatever in the spring following inoculation; cankers arose on the one-year-old branches, half to three-quarters girdling and therefore not killing the branches.

Expt. 26/32. Using strains from stem lesions inoculations were made on three-year-old plum branches; with each strain two branches were inoculated at two places each (eight inoculations in all, with controls). Cankers arose at all inoculated points. One branch became girdled and died; another was nearly girdled and bore yellowish curled leaves during the summer. One control branch showed slight disturbance but the others all healed normally.

Expt. 27/8. Eight strains were used, five from stem lesions and one each from leaf, shoot and fruit lesions. The inoculations were made on April 25th on the opening leaf buds of one-year-old twigs; eight buds were inoculated on each twig by placing on them drops of the bacterial suspension in water, and alternate buds were then punctured. With two stem strains four twigs were inoculated in this way; two twigs were used for each of the other strains; thus twenty twigs (160 buds) were inoculated, half the buds being punctured. The trees were examined on May 9th when the buds had unfolded; forty-eight (or 30 per cent.) of the inoculated buds showed infection on one or more leaves. The infection spots were most numerous on the leaves developing from punctured buds, but some of the unwounded buds also bore infected leaves; the infections appeared as irregular blackened lesions or as circular spots. Ten control twigs, with buds similarly treated but inoculated with sterile water only, showed no infection; the punctures that had been made in these buds now showed as holes in the leaves but there was no blackening of the tissues.

Expt. 27/24. Inoculations were made on November 7th, 1927, using three strains; two of these had been re-isolated in 1926 from shoots inoculated in the summer with pure cultures, and the other was re-isolated from one of the infected trees of Expt. 26/21. On tree No. 1 each strain was inoculated into four twigs; on tree No. 2 each was inoculated into two two-year-old branches, two inoculations being made on each branch.

All the inoculations produced cankers. On tree No. 1 six twigs were girdled and killed; on tree No. 2 four of the six inoculated branches were killed.

Remarks on the Inoculation Experiments

The striking results of Expt. 26/21 offer convincing proof that the bacterium isolated from stem lesions is the primary cause of the cankers, the symptoms arising being similar to those observed in naturally infected trees. The actual damage was caused during the months October to April, and as soon as the outlines of the cankers were observed (in May) there was no further increase in the size of

the lesions either in that year or in subsequent years. Although various fungi appeared on the bark of the cankered areas they were unable to extend beyond the limits of the original cankers, thus showing that they were secondary, living on the tissues killed by the bacteria.

Expts. 26/22, 26/29 and 26/31 are interesting in that they confirm the results of cultural tests which go to show that the same organism is able to infect stems, leaves, shoots and fruit. The identity of the organism found in leaf spots with that of the stem lesions is important in relation to the annual cycle of the organism, which apparently spends the winter months in the stems and branches producing lesions which appear as cankers in summer, while during the summer it exists on the young green parts of the trees, particularly on the leaves.

Expt. 26/22 showed that cankers which do not girdle the stems before May do not do so later, but tend to become covered with callus, and the tree survives. This is in conformity with observations on naturally infected trees.

It is obvious that the organism cannot be regarded as a "weak parasite," for during the few months when it is active in the stems it may produce cankers from two to three feet long.

Description of the organism causing bacterial canker of plum trees

The bacterium used in the inoculation experiments just described shows relationship with *Pseudomonas prunicola*, which was found to be the cause of a Shoot Wilt in plum trees (2). The two differ however in certain characters, as indicated in the following description of *Pseudomonas mors-prunorum*.

Morphology. The organism is a rod-shaped bacterium with rounded ends. In smears prepared from two-day-old cultures on nutrient agar, fixed with formalin and stained with methyl violet, the rods measured $0{\cdot}8 - 2{\cdot}5 \times 0{\cdot}3 - 0{\cdot}5\mu$, in those not showing constrictions; the rods were mostly single or double; constricted rods only $2{\cdot}0$ to $2{\cdot}5\mu$ long were frequently seen. They were rarely connected to form long filaments; the longest filament seen was 25μ long.

The rods stain well with carbol fuchsin, gentian violet, aniline gentian violet, and methyl violet, less deeply with bismarck brown, and faintly with methylene blue. They are stained slightly by Gram's stain, by the method given by Eyre* and by the "usual procedure"† using aniline gentian violet or carbol gentian violet.

Stained by Plimmer and Paine's method the rods mostly showed

* *Bacteriological Technique*, 2nd ed. 1913, p. 108.

† *Manual of Methods*, Society of American Bacteriologists, Supplement A, p. 7.

one polar flagellum, two to three times the length of the rod, but rods with two or three flagella were frequent.

No endospores have been seen; the growth taken from nutrient agar slopes, seven days old, and heated at 80° C. for ten minutes, was killed, thus confirming the absence of spores.

Capsules have not been clearly demonstrated but, when stained by Plimmer and Paine's flagella stain, many rods showed a hyaline "sheath" with the flagella attached to the sheath.

Temperature Relations. The thermal death point of the organism is 46° C. (heating for ten minutes), but lower temperatures, *e.g.* 40°, 42° and 44° C., had a retarding effect on the development of the organism, for when tubes after being heated at those temperatures were restored to more normal conditions there was delayed growth.

The rate of growth has been compared for temperatures 15°, 20°, 25°, 30° and 35° C. At 25° C. the growth was generally a little more rapid than at 20° C. during the first twenty-four hours, but from two days onward no constant difference could be detected. The lower (15° C.) and the higher (30° C.) temperatures showed a distinct retarding effect on development during the first week, but later there was little difference. The optimum temperature for growth is therefore round about 25° C. Tubes inoculated and kept at 35° C. showed no growth whatever, and on being restored to room temperature at the end of nine days no turbidity appeared.

It was evident then that a temperature of 35° C. not only inhibits the development of the organism but has a lethal effect. To test this further, twenty-four tubes of nutrient broth with saccharose were inoculated (using three strains, eight tubes for each) and incubated at 35° C. Two tubes from each batch of eight were removed, and placed at room temperature, at intervals of five, seven, nine and ten days from the time they were placed in the incubator. No turbidity appeared in any of the tubes while in the incubator. After being restored to room temperature, two of those which had been incubated for five days became turbid, while all the rest remained sterile. Control tubes, inoculated and kept at room temperature from the beginning of the experiment, showed some turbidity after twenty-four hours and developed normally. The results showed that a temperature of 35° C. not only inhibited the development of the inoculum but destroyed it in five to seven days.

Cultural Characters

The organism produces a greyish or almost colourless growth on nutrient agar slopes, but on this medium containing 5 per cent. saccharose the colour is white. On potato plugs there is a creamy yellow tinge, but the general colour of the organism on agar and in

broth is not that of a yellow organism and it is classified therefore as white (non-chromogenic).

It is strongly aerobic, as shown by the absence of clouding in the closed arm of fermentation tubes, and by its tendency to form pellicles on the surface of liquid media.

The characters noted below are for cultures at 25° C. unless otherwise stated.

Nutrient broth (Difco). Growth is rather weak as the medium never becomes very turbid. When used with an indicator an alkaline reaction is shown, the colour change becoming noticeable in two days after inoculation.

Beef extract (from fresh meat). Growth is rather weak; there is little change in colour and in this the organism differs from *Ps. prunicola* which turns this medium yellow.

In nutrient broth containing dextrose, lactose, saccharose (1 per cent.) or glycerin (1 or 2 per cent.) there is good growth; in Durham's tubes there is no growth in the inner (inverted) tube and no gas appears.

Dextrose litmus broth. Growth is more vigorous than in broth without sugar; there is a definite acid reaction within a few days.

Lactose litmus broth. The reaction after a few days is alkaline and generally remains so, but with some strains it is reversed and becomes acid.

Saccharose litmus broth. The reaction is generally acid but often masked by a yellowish coloration.

Glycerine litmus broth. With most strains tested the reaction is acid eventually.

Indicator broth. As the litmus-containing cultures sometimes produced a yellowish coloration tending to mask the colour change of the litmus, series of cultures were started using the sugars mentioned above and glycerin in broth cultures containing the sulphonphthalein indicators bromo-cresol purple and cresol red in combination. Nutrient broth alone showed an alkaline reaction in about three days, with no reversal of the reaction. In dextrose broth there was an acid reaction in two to three days with most strains, the acidity becoming pronounced by about the seventh day. In lactose broth the reaction was in the alkaline direction, but it was not so pronounced as in corresponding tubes of *Ps. prunicola*. In saccharose broth there was usually acidity showing in three days and it was well marked within seven days. In glycerin broth there was an alkaline reaction up to about the fifteenth day when there was a reversal, the change towards acidity taking place more rapidly with some strains than with others. With one strain (from a fruit spot) the cultures remained alkaline for about eight weeks but eventually an acid reaction was clearly shown.

It would appear from these tests that the organism is able to produce acid from dextrose, saccharose and glycerin but not from lactose. Results obtained from cultures on "purple lactose agar" (see below) showed, however, that on this medium lactose also is a source of acid. The discrepancies are doubtless due to the alkalinity that arises from the action of the organism on the broth (containing peptone); this would tend to neutralise any acid derived from the lactose. By increasing the concentration of the lactose to 2 per cent. it was found that acid production could be detected, although with some strains the reversal to acidity took place very gradually.

In broth containing 5 per cent. lactose there was again always an alkaline reaction at first; this persisted for some time, but a reversal towards acidity began to appear in from two to eight weeks, the time varying with the individual strains. Sometimes the reaction was still on the alkaline side of neutrality after twelve weeks. The final tests for acidity were made (by the capillator method) when the cultures were twenty weeks old, and the *p*H was found to be from 4·5 to 4·9; control tubes (incubated along with the inoculated tubes) gave *p*H 6·0 at the end of the experiment, and corresponding cultures of *Ps. prunicola* gave *p*H 7·2. This variability in the production of acid from lactose, according to the strains used, probably explains the variability observed in milk cultures (described below), cow's milk containing about 4·5 per cent. lactose.

Nutrient broth + 5 per cent. saccharose. There is good growth in this medium, which becomes turbid within a few days; a pellicle is produced which tends to fragment easily, and there is a thick sediment eventually. The white (or opalescent) turbidity is another character serving to distinguish the canker organism from *Ps. prunicola*, which in this medium produces a more translucent growth giving a yellowish tinge to the liquid. The difference is most easily seen when tubes of the two organisms are held side by side in front of a dark background.

Uschinsky's solution. In this medium using the acid phosphate (KH_2PO_4) without adjustment, giving a medium of *p*H 5·5 after sterilisation, there was no growth; when it was neutralised with NaOH, giving a *p*H of 6·4 after autoclaving, there was fair growth, with a pellicle. No yellowing of the medium appeared such as occurred in parallel cultures of *Ps. prunicola*. A similar result was obtained when the medium was prepared with the basic phosphate, K_2HPO_4, without adjusting (*p*H 6·8 after autoclaving).

Nutrient broth + nitrate. There was no reduction of nitrate to nitrite in nutrient broth containing 0·1 per cent. potassium nitrate.

Tryptophane broth. Growth was fair; a fragile pellicle was produced; cultures tested for indol (Ehrlich-Böhme's test) when fourteen days old gave negative results.

Cohn's solution. There was no growth in this medium.

Milk. Cultures in milk gave variable results according to the strains used; this probably bears some relation to the amount of acid produced from the lactose by the different strains.

In some plain milk cultures there was no visible change, compared with control tubes, after several weeks, except that a sediment appeared showing that growth had taken place. In others a definite solid curd was produced so that the tubes could be inverted without the contents becoming dislodged, but sometimes a layer of whey appeared above.

Litmus milk. There is a reaction in the alkaline direction, usually showing first towards the upper end of the medium, but there is not the definite zoning shown by *Ps. prunicola*. The litmus tends to become decolorised so that this medium is not so useful as the next for showing reversal of the reaction.

Milk with bromo-cresol purple as indicator. There is a definite alkaline reaction in a few days, but after two or three weeks there is usually a reversal, the appearance of a yellow tint indicating a *p*H on the acid side of neutrality. In those cultures where a solid curd was formed the reaction was definitely acid, but acidity was not always accompanied by curdling.

Methylene blue milk. This medium becomes decolorised within a few days; shaking (by rolling in the hands) restores the colour to some extent, but decoloration again sets in. On heating the tubes in boiling water for two minutes and allowing to cool, the blue colour reappears and persists.

Nutrient agar. In poured plates the colonies come up rather slowly, and generally they can only just be seen with the naked eye in forty-eight hours. By the sixth day they are about 2 mm. in diameter, circular, greyish, with no characteristic structure except that faint wavy lines can sometimes be made out under low magnification. On slopes the growth is grey, only slightly raised and therefore forming a rather thin layer.

Nutrient agar + 5 *per cent. saccharose.* This medium was found useful for distinguishing *Ps. prunicola* from accompanying saprophytes and was used with success in isolating and identifying the canker organism. In plate cultures the colonies bear a resemblance to those of *Ps. prunicola*, but those on the surface are more nearly white; they are more highly refractive and glistening and more raised, becoming almost, or quite, hemispherical. The radial markings on the surface, embedded and bottom colonies are very much like those of *Ps. prunicola*, when examined from below under a low power of the microscope. In slant cultures the growth is white, glistening and raised.

Purple lactose agar (nutrient agar + lactose, containing bromo-cresol purple as indicator). This medium as usually prepared contains 1 per

cent. lactose. In the earlier tests the medium (as slopes) showed an alkaline reaction in about twenty-four hours; the blue-purple colour became more intense and then after some days the reaction was reversed; a yellow coloration appeared at the upper end of the slope and gradually extended throughout the medium to the base. At the time, this appeared to be a critical test for distinguishing this organism from *Ps. prunicola*, which invariably produced an alkaline reaction without any change in the acid direction. Later, however, certain strains of the canker organism failed to give the acid reaction on the medium as generally used, but it was found that, by increasing the lactose to 2 per cent., these strains also produced the acid in sufficient amount to change the blue-purple of the medium to yellow, while *Ps. prunicola* on the same medium failed to show any acid reaction. Purple lactose agar containing 2 per cent. lactose therefore is now used as a test for distinguishing the two organisms.

Nutrient gelatin. Cultures on nutrient gelatin have yielded variable results, some strains liquefying it, others not. When liquefaction occurs it usually progresses more slowly than with *Ps. prunicola.* In plate cultures the surface colonies are circular, with a dense central "nucleus"; the embedded colonies are spherical and brownish.

Nutrient gelatin + 5 *per cent. saccharose.* In plate cultures the young surface colonies (two days old) are subcircular; under a low power of the microscope irregular lines suggesting reticulation may be seen. The embedded colonies are subspherical but may appear dentate in optical section; they are almost without definite structure but may show traces of reticulation. If no liquefaction takes place the surface colonies become very raised (approximately hemispherical).

Potato plugs. Within three days there is a creamy yellow growth along the streak, the rest of the medium developing a blue-grey colour.

Media containing starch. No clear evidence has been obtained that the organism reduces starch. Nutrient broth cultures containing starch (0·2 soluble starch or 0·03 rice starch), tested when four weeks old, sometimes gave an evanescent reddish colour on adding one to three drops of iodine solution (Lugol's), which may indicate a slight action on starch, but on adding excess of iodine the inoculated tubes gave a starch reaction as pronounced as the control tubes.

Stroke cultures on nutrient agar + 0·2 per cent. soluble starch were flooded with iodine, some when one week old, others two weeks old; the starch was cleared in a very narrow zone immediately round the growth and in the agar below the growth, but there was no clearing of starch beyond those limits, so that there would appear to be no secretion of a diastasic enzyme.

Longevity in Culture Media

With regard to broth and nutrient agar media containing saccharose, it has been found that, although the organism makes far more vigorous growth when the sugar is added, its period of viability is reduced. This is very marked in nutrient agar containing 5 per cent. saccharose. Stroke cultures on this medium grow rapidly, forming a dense, white, raised growth, within four days, and then die. In many tests transfers from such cultures when four days old, to nutrient broth, have given no growth, while stroke cultures on nutrient agar without sugar remain viable for several weeks. It is advisable, therefore, when preparing isolation plates using nutrient agar with 5 per cent. saccharose, that sub-inoculations from colonies should be made by the third day at the latest, particularly if the colonies come up quickly on rather thickly sown plates.

Cultures in nutrient broth + 5 per cent. saccharose become more turbid than in nutrient broth without sugar, but die out more quickly. Some strains have been lost by keeping them in nutrient broth with saccharose more than five weeks before subculturing. In order to keep the organism alive and vigorous for longer periods, nutrient broth + 2 per cent. glycerin is now used; cultures six months old in this medium have been found to be viable.

The early dying out of cultures in media with a relatively high concentration of saccharose is correlated with the rapid production of acid, and it is probably this acid, produced by the organism itself from the sugar, that has a toxic effect. Slope cultures of nutrient agar + 5 per cent. saccharose, tinted with bromo-cresol green as indicator, showed an acid reaction of about *p*H 4·2 within four days.

Comparison with Ps. prunicola

Pseudomonas mors-prunorum, the organism causing bacterial canker of plum trees, shows affinity with *Ps. prunicola* (2), but the two differ in certain particulars, which have been referred to in passing in describing *Ps. mors-prunorum*. For ready reference the more striking cultural differences may be tabulated as follows:

Culture medium	*Ps. mors-prunorum*	*Ps. prunicola*
Nutrient broth + 5 per cent. saccharose	White cloudy growth	Growth yellowish, more translucent
Nutrient agar + 5 per cent. saccharose	Rapid production of acid; growth usually dead in four to six days	Less rapid acid production; growth remains viable for several weeks
Nutrient agar + 2 per cent. lactose with bromo-cresol-purple as indicator	Reaction at first alkaline, later acid, medium turning yellow	Reaction alkaline, the medium becoming blue-purple and remaining so
Uschinsky's solution	Yellow coloration absent or very faint	Distinct yellow coloration

Fig. 3

Fig. 2

Fig. 1

Fig. 6

Fig. 5

Fig. 4

The "Group Number" of *Ps. mors-prunorum* is 211·2222032. Its "Index Number," showing the "Primary Characters" according to the "Descriptive Chart" of the Society of American Bacteriologists, is 5021—31100—0222. According to Erwin F. Smith's classification it would be in the genus *Bacterium* (Cohn emend.), while in the recent classification adopted by the Society of American Bacteriologists its name would be *Phytomonas mors-prunorum*.

Summary

The chief cause of Die-back in plum trees is Bacterial Canker.

Inoculation experiments are described showing that Bacterial Canker in plum trees is brought about by an organism which has been isolated not only from stem cankers but also from lesions on shoots, leaves and fruit.

Details are given of the morphology and cultural characters of the organism, which has been named *Pseudomonas mors-prunorum*.

EXPLANATION OF PLATES VII AND VIII

Figs. 1, 2 and 3 illustrate Expt. 26/21. In each the tree on the right was inoculated with *Pseudomonas mors-prunorum* (isolated from a stem lesion) on October 4th, 1926; the one on the left is a control tree. Photographed July 29th, 1927.

Fig. 1. Inoculated tree killed nearly to base of scion; the buds on that portion of the scion below the canker have grown out to form healthy shoots.

Fig. 2. Inoculated tree killed to base of scion; shoots are growing up from the stock.

Fig. 3. Inoculated tree killed outright.

Fig. 4. (Expt. 26/29.) Branch on left inoculated with *Ps. mors-prunorum* isolated from a leaf-spot; control branch on right.

Fig. 5. (Expt. 26/31.) Twigs inoculated November 19th, 1926, with strains from shoot lesions.

Fig. 6. (Expt. 26/31). Twig on left inoculated November 19th, 1926, with a strain from a fruit lesion; control twig on right.

The photographs for Figs. 4, 5 and 6 were taken on May 18th, 1927.

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A LIST OF THE ENTOMOGENOUS FUNGI OF GREAT BRITAIN

By T. PETCH

The following list includes the entomogenous fungi (excluding the Laboulbeniomycetae) which have been previously recorded as occurring in Great Britain, and others which have been added in the years 1930, 1931. The latter are indicated by an asterisk.

ENTOMOPHTHORACEAE

Empusa Muscae Cohn in *Nova Acta Acad. Caes. Leopold.-Carol. Germanicae Nat. cur.* xxv (1855), Abh. 1, 301; ? *Sporendonema Muscae* Fr., *Syst. Myc.* III, 435 (1832).

Recorded by Berkeley in *English Flora*, VI, 350, "On flies, adhering to leaves, ceilings, etc. Autumn. Extremely common. This is the white substance, noticed in *Loudon's Mag. Nat. Hist.* v. 7, p. 532." "Common on flies in the autumn," Cooke, *Handbook* (1871), p. 639. Scarborough, Halifax, Masham (*Fungus Flora of Yorkshire*, 316). Kirbymoorside, August 1910 (Y.N.U. Excursion). Worcester, 1912; Baslow, 1919 (Brit. Mycol. Soc. Forays).

On a fly on Nettle, Harford Bridge, Norwich, June 1925! On Cabbage Root fly, Leeds, June 1930 (T. H. Taylor)! On fly on Bramble flowers, Houghton Woods, East Yorks., July 1930! Holt House Wood, near King's Lynn, June 19, 1931! On flies on *Rumex*, *Senecio*, *Juncus*, *Anthriscus*, etc., Hornsea, Aldbrough, and Hedon, abundant, July 1931! On fly on *Rumex*, Shipley, July 1931 (A. M. Smith)! North Wootton, Norfolk, August 27, 28, 1931! On flies on grasses, Kendal Fell, Westmorland, August 28, 1931 (E. W. Jones)! Grassington, September 1931! See also *Empusa Culicis* and *Empusa conglomerata.*

**Empusa Culicis* A Braun, *Algarum unicell.* nov.gen. (1855), p. 105.

[Recorded by Cooke in *Gardeners' Chronicle*, Ser. 3, XVIII (1895), 620, 685, on flies on ears of Barley, preserved by Henslow in 1840, but the specimens, now in Herb. Kew., are *Empusa Muscae.*]

On a gnat, Hornsea, July 16, 1931! On a small fly, Holt House Wood, near King's Lynn, August 17, 1931!

Empusa conglomerata (Sorokin) Thaxter, *Mon.* p. 162 (1888).

[Recorded by Cooke in *Gardeners' Chronicle*, Ser. 3, XVIII (1895), 18, 135, on *Melanostoma scalare*, Blackroot Bog, Sutton Coldfield; and on the same host, Lyndhurst, New Forest, by Miss A. L. Smith in *Trans. Brit. Mycol. Soc.* III (1909), 111. Both specimens are *Empusa Muscae.*]

**Empusa Aulicae* Reichhardt in litt. (cf. Bail in *Schrift. d. naturf. Gesellsch. zu Danzig*, n.f., II (1869), 3).

On a caterpillar, Reffley Wood, King's Lynn, June 8, 1930! (entered in error for the Whitby Foray of 1930 in *Trans. Brit. Mycol. Soc.* XVI (1931), 15). On caterpillars on grass, Ben Lawers, August 1931 (E. W. Jones)!

**Empusa Tipulae* (Fres.); *Entomophthora Tipulae* Fres. in *Abhandl. d. Senckenberg. naturf. Gesellsch.* II (1858), 206.

On a Tipulid hanging from a dead log, Bishop's Wood, Selby, July 11, 1931! On a Tipulid, Oakdale, Osmotherley, August 1931 (F. A. Mason)!

Empusa Fresenii Nowak. in *Proc. Krakow Acad. Sci.* (1883), p. 153.

Recorded by A. D. Cotton in *Trans. Brit. Mycol. Soc.* VI (1919), 202, on aphids on Gooseberry, Swordale, Evanton, Ross-shire, August 1918, and on aphids on Potato, Stirkoke, Wick, September 9, 1918, collected by D. J. Jackson.

On aphis on Meadowsweet, Hornsea, July 14, 1931!

**Empusa Planchoniana* (Cornu) Thaxter, *Mon.* p. 165 (1888).

On aphis on Birch, Shouldham Warren, Norfolk, June 17, 1930! On aphis on Bramble, Holt House Wood, near King's Lynn, June 19, 1931! October 17, 1931! On ? hemipteron, Hornsea, July 14, 1931! On aphis on Meadowsweet, Aldbrough, East Yorks., July 17, 1931!

**Empusa papillata* Thaxter, *Mon.* p. 166 (1888).

On a fly, North Wootton, King's Lynn, August 26, 27, 1931! On a fly, Hagg Wood, near Burnsall, September 15, 1931!

**Entomophthora Lampyridarum* Thaxter, *Mon.* p. 169 (1888).

On beetles (*Agriotes*) attached to grasses by their mandibles, between Coton and Hardwick, Cambridge, May 3, 1931 (E. W. Jones)!

Entomophthora Aphidis Hoffm. in Fres. *loc. cit.* 208; *Entomophthora ferruginea* Phillips in *Ann. Mag. Nat. Hist.* Ser. 5, XVIII (1886), 4.

Recorded by Cooke in *Gardeners' Chronicle*, Ser. 3, XVIII (1895), 266; also by A. D. Cotton in *Trans. Brit. Mycol. Soc.* VI (1919), 201, on aphis on Gooseberry, September 18, 1917, and on aphis on *Allium* and Gooseberry, August 12, 1918, Swordale, Evanton, Ross-shire, coll. D. J. Jackson.

On aphis on Nettle, Leyburn, May 23, 1931! On aphis on Meadowsweet, Hornsea, Aldbrough and Hedon, East Yorks., July 1931! On *Aphis rumicis*, Yorkshire, August 1931 (T. H. Taylor)! On aphis on *Prunus*, Burnsall, September 16, 1931! On aphis on Elm, Bolton Woods, September 17, 1931! On aphis on Bramble, Holt House Wood, near King's Lynn, October 17, 1931!

Entomophthora sphaerosperma Fres. in *Bot. Zeitung*, XIV (1856), 882.

On a caterpillar on grass, Rushton, Somerset, coll. W. Watson, May 1918 (A. D. Cotton, *Trans. Brit. Mycol. Soc.* VI (1919), 202). On *Scatophaga merdaria*, Bradford, Yorks., June 1921 (F. A. Mason, *Naturalist*, September 1927, p. 272).

On flies, Hornsea, July 7, 1930! July 14, 1931! Holt House Wood, near King's Lynn, June 19, 1931! August 17, 1931! North Wootton, Norfolk, August 26, 27, 1931! Burnsall, September 16, 1931! Bolton Woods, September 14, 17, 1931!

On aphis on Nettle and *Aspidium*, Bolton Woods, September 17, 1931! On aphis on Bramble, Holt House Wood, near King's Lynn, October 17, 1931!

On frog-hoppers (*Philaenus spumarius*), Arncliffe Woods, near Whitby, August 1930 (F. A. Mason)! Grassington, September 12, 16, 1931!

On beetle, Hornsea, July 14, 1931!

On an ichneumon, North Wootton, Norfolk, September 8, 1931!

Entomophthora muscivora Schroet. in *Krypt.-Flora v. Schlesien*, III, pt. 1, 223 (1886).

On a fly on a dead stoat, Aldbrough, East Yorks., October 1925! (*Naturalist*, 1925, p. 368).

Entomophthora americana Thaxter, *Mon.* p. 179 (1888).

On flies, often on tree trunks, or on the under side of dead branches, rotting logs, or wood, lying on the ground, or on clay banks by roadsides.

On an Anthomyid (*Hyetodesia erratica*, Fabr.) on the trunk of a tree, Epping Forest (H. Main, *Essex Naturalist*, XVIII (1917), 107), det. J. Ramsbottom.

Arncliffe Woods, Osmotherley, August 1931 (F. A. Mason)! Burnsall, September 16, 1931! Hagg Wood, Burnsall, September 15, 1931! Sutton Park, Warwickshire, September 20, 1931 (E. W. Jones)! North Wootton, October 24, 1931! On a leaf-hopper, Holt House Wood, near King's Lynn, August 17, 1931!

**Entomophthora variabilis* Thaxter, *Mon.* p. 183 (1888).

On flies, Holt House Wood, near King's Lynn, August 17, 1931! West Briggs, near King's Lynn, August 21, 1931! North Wootton, Norfolk, August 27, 1931!

**Entomophthora dipterigena* Thaxter, *Mon.* p. 177 (1888).

On flies, Holt House Wood, near King's Lynn, August 17, 1931! October 17, 1931 (abundant)! West Briggs, near King's Lynn, August 21, 1931! Burnsall, September 13, 1931! North Wootton, Norfolk, September 8, 1931! October 24, 1931 (abundant)! On a Tipulid, Bolton Woods, September 17, 1931.

**Entomophthora echinospora* Thaxter, *Mon.* p. 180 (1888).

On flies, North Wootton, Norfolk, August 26, 1931! September 8, 1931! Holt House Wood, near King's Lynn, October 17, 1931!

The body of the insect breaks up, and the fragments fall away, leaving an ashy or pale yellow heap of resting spores with sometimes only part of a leg to indicate the former presence of a fly.

**Entomophthora* (*Tarichium*) *Lauxaniae* Bubák in *Hedwigia* (1903), p. 100.

In a fly attached to a head of *Eriophorum*, Derby Fen, near King's Lynn, July 18, 1930!

**Entomophthora* (*Tarichium*) *atrosperma* Petch, n.sp.

In an aphis, Grassington, September 12, 1931 (F. A. Mason)!

Sporis perdurantibus corpus pulvere nigro implentibus, nigro-brunneis, globosis, 38–45μ diam., spinis crassis conicis, 2μ longis, ornatis; insectum ad substratum hyphis paucis hyalinis affixum.

**Entomophthora* (*Tarichium*) *Coleopterorum* Petch, n.sp.

In a beetle, Holt House Wood, near King's Lynn, June 12, 1931!

Sporis perdurantibus corpus pulvere fusco-brunneo implentibus, fusco-brunneis, episporio crasso (ad 6μ), dense verrucosis, globosis, 35–50μ diam., vel late ovalibus, 48–52 × 44–46μ, vel interdum pyriformibus, 56 × 44μ; insectum ad substratum hyphis fasciculatis rhizoideis ex abdomine oriundis affixum.

The resting spores of *Entomophthora Lampyridarum* are unknown, but the mode of attachment of the insect to the host plant is quite different in that species.

HYPOCREACEAE

Cordyceps militaris (L.) Link, *Handb.* III, 347 (1833); *Sphaeria militaris*, Bolton, Pl. 128 (1789); Sowerby, Pl. 60 (1797); Purton, *Midland Flora*, III, 276, Pl. 23 (1821); Berkeley, *Eng. Flora* V, pt. 2, 232 (1836); *Cordiceps militaris* Fr., Berkeley, *Outlines*, Pl. 23, fig. 4 (1860); *Torrubia militaris* Fr., Cooke, *Handbook*, p. 770 (1871); *Cordyceps militaris* Linn., Cooke, *Vegetable Wasps, etc.* p. 173, Pl. 2, fig. 15 (1892).

Hill's record in *Flora Britannica*, p. 653 (1760), was based on Ray, *Synopsis*, ed. 3 (1724), p. 14, "*Fungoides clavatum*," etc., which refers to a yellow *Clavaria* found on Hampstead Heath; hence Hill's reference is incorrect. Hudson's record in *Flora Anglica* (1778), p. 638, was based on Ray, *Synopsis*, p. 12, "*Fungus fontanus purpureus elegans*," which was apparently a *Claviceps*, growing on floating grasses. Relhan, *Flora Cantabrigiensis*, Supplement, p. 33, No. 1054 (1786), followed Hudson and Ray, giving as a habitat, Gamlingay Bogs, which is Ray's locality for *Fungus fontanus purpureus elegans*, though he added correct details concerning the dimensions and colour of *Cordyceps militaris*, probably from *Flora Danica*.

Sowerby (*loc. cit.*) recorded *Sphaeria militaris*, "on the unquickened remains of a spider's nest." That record has not been duplicated, though I have collected a *Spicaria* on a spider's cocoon.

Purton (*op. cit.*), p. 278, stated: "We find them in great plenty this year (June 1820) in Ragley Woods; and every specimen was attached to a chrysalis."

Berkeley in *English Flora* (*loc. cit.*) wrote: "In wood among moss, arising

from dead pupae of insects, spiders' eggs, etc. Summer. Not common, but sparingly diffused through the whole of England and Scotland."

On lepidopterous larvae and pupae. Generally distributed but not common. Usually recorded in September. Specimens on lepidopterous larvae, Lees (Lancs.), September 1930 (F. J. Stubbs)!

Cordyceps gracilis Mont. & Dur., *Flor. Alger.* I, 449, Pl. 25, fig. 2 (1846–49); *Sphaeria entomorrhiza* Berk. non Dicks., *Eng. Flora*, V, pt. 2, 232 (1836); *Cordiceps entomorrhiza* Berk., *Outlines*, p. 382, Pl. 23, fig. 5 (1860); *Torrubia entomorrhiza* Fr., Cooke, *Handbook*, p. 769, fig. 367 (1871); *Cordyceps entomorrhiza* Cooke, *Vegetable Wasps, etc.*, p. 164 (1892); *Cordyceps Mawleyi* Westwood in *Gardeners' Chronicle*, Ser. 3, IX (1891), 553, fig. 115.

I have omitted reference to *Xylaria gracilis* Grev., because it would appear, from a specimen from Greville in Herb. Kew., that Greville's species is *Cordyceps capitata* (Holms.).

On larvae of lepidoptera, usually *Hepialus*. Spring. This species has generally been recorded in this country as *Cordyceps entomorrhiza*.

Edgefield, Norfolk (Berkeley). Kirby Bedon, Norfolk, April 1853 (K. T.)! North Wootton, Norfolk, May 1871, May 1873 (Plowright)! Apethorpe, Northants, May 17, 1835 (Berkeley)! Thringstone, April 1, 1836 (C. Babington)! Henlow, Beds., April 1866 (Cooke)! Berkhamsted (E. Mawley). Hartham Park, Wilts., April 29, 1843 (Broome)! Southborough, Tunbridge Wells (W. Fawcett)! Bognor, 1908 (E. R. Bankes)! Leigh Court, Somerset (Broome)! Near Coddington, Cheshire, May 3, 1836 (J. E. B.)! Higher Greenwood, Hebden Bridge, May 6, 1893 (N. & P.)! Masham, Yorks., April 27, 1902 (W. A. Thwaites)!

Cordyceps entomorrhiza (Dicks.) Link, *Handb.* III, 347 (1833). *Sphaeria entomorrhiza* Dickson, *Plant. Crypt. Brit.* I, 22, Pl. 3, fig. 3 (1785). *Xylaria entomorrhiza* (Dicks.) Gray, *Nat. Arrang. Brit. Plants*, I, 511 (1821). Non *Sphaeria entomorrhiza* Berk., *English Flora*, V, pt. 2, 232 (1836). *Torrubia cinerea* Tul., *Sel. Fung. Carp.* I, 61, Pl. 1, fig. 11 (1861). Lloyd, *Letter* 47, pp. 15, 16; 51, p. 4.

On a beetle larva, Bulstrode, Bucks., in the autumn (Dickson), the only British record, others being usually *Cordyceps gracilis*.

**Cordyceps sphecocephala* (Kl.) Cooke, *Vegetable Wasps, etc.* p. 40 (1892).

Specimen on a hornet in Herb. British Museum, in the cover of *Cordyceps entomorrhiza*, marked "*Sphaeria* from the Rev. G. Salmon," *ex* Herb. Broome, without date or locality!

To this species is probably to be referred the record of *Torrubia entomorrhiza*, on a wasp in moss at Sluie, by the Rev. J. Keith, in *A list of the fungi hitherto found within the province of Moray*, *Scottish Naturalist*, 1874.

Cordyceps Forquignoni Quélet in *Assoc. Franc. p. l'Avancement d. Sciences*, 1887, p. 6, Pl. XXI, fig. 18. *Hypocrea myrmecophila* B. & Br. non Ces. in *Ann. Mag. Nat. Hist.* Ser. 2, VII (1851), 186.

On flies, usually recorded in Britain as *Cordyceps myrmecophila*. On a fly (not Ichneumon), Leigh Wood, Somerset, May 1846, 1847 (C. E. Broome)! Trefriw, North Wales, May 1874 (Phillips)! Higher Greenwood, Hebden Bridge, May 6, 1893 (N. & P.)! Masham, August 8, 1902 (W. A. Thwaites)! Dolgelly, B.M.S. Spring Foray, 1913. Bettwsycoed, B.M.S. Foray, 1924. Grassington, September 16, 1931 (F. A. Mason)!

Cordyceps clavulata (Schw.) E. & E., *North Amer. Pyreno.* 61, Pl. 15 (1892); *Sphaeria clavulata* Schw., *Syn. N.A.F.* No. 1155 (1831); *Cordyceps pistillariaeformis* B. & Br. in *Ann. Mag. Nat. Hist.* Ser. 3, VII, 451, Pl. 16, fig. 22 (1861); Cooke, *Vegetable Wasps, etc.* p. 317, Pl. 2, fig. 19 (1892); *Torrubia pistillariaeformis* Cooke, *Handbook*, p. 771 (1871).

On a scale insect on Wych Elm, Batheaston, Somerset, March 1860, October 1860 (Broome)!

MYRIANGIACEAE

Myriangium Duriaei Mont. & Berk. in *Hooker's London Journ. Bot.* IV (1845), 73.
In Britain, usually found on *Chionaspis salicis* on Ash.
Trevella Wood, Penzance, 1864 (W. Curnow)! December 10, 1873 (W. C.)!; Trevella Carne Grove, Penzance, March 28, 1865 (W. C.)! Treventh Farm, Penzance, 1870 (W. C.)! Treventh, near Penzance, March 7, 1871 (W. C.)! Castle Hornock, Penzance, February 1870 and January 1871 (W. C.)! St Levan, May 1874 (W. C.)! Trengwainton, Penzance; and others in Herb. B. M.
Ilsham Walk, Torquay (J. M. C.)! Shanklin, Isle of Wight, April 1873 (J. M. C.)! Cook's Castle, near Shanklin (H. Piggot)! Near Ryde, Isle of Wight (A. B.)! Brading, Isle of Wight, 1868! Danny Park, West Sussex (W. Mitten)! Horner Woods, near Minehead, Somerset, September 1920! Cleeve Combe, Somerset, September 1923 (Brit. Mycol. Soc.). Hafod Woods, Bettwsycoed, September 1924 (Brit. Mycol. Soc.).

MUCEDINACEAE

**Monilia penicillioides* Delacr. in *Bull. Soc. Myc. France*, XIII (1897), 114.
On pupae of the Cinnabar Moth from Farnham Royal, May 1931!

Cephalosporium Lefroyi Horne in *Gardeners' Chronicle*, LVII (1915), 139.
On *Aleyrodes vaporariorum*, Wisley.

Cephalosporium (*Acrostalagmus*) *coccorum* Petch, in *Trans. Brit. Mycol. Soc.* X (1925), 175.
On *Chionaspis salicis* on Ash, Aldbrough and Hedon, East Yorks., September 1920! on *Lepidosaphes ulmi* on Hawthorn, Hunstanton, October 1920–February 1921! on *Lepidosaphes* on Apple, Camberley, November 1920 (E. E. Green)!

Cephalosporium (*Acrostalagmus*) *Eriophytis* (Massee) Petch in *Trans. Brit. Mycol. Soc.* XVI (1931), 66; *Botrytis Eriophyes* Massee in Taylor, *Journ. Econ. Biol.* IV (1909), 5; *Verticillium Eriophytis* (Massee) Sacc & Trott., *Syll. Fung.* XXII (1913), 1299.
On *Eriophyes ribis* in "big bud" of Black Currant (type in Herb. Kew.)! North Wootton, King's Lynn, April 1932!

**Cephalosporium* (*Acrostalagmus*) *muscarium* Petch in *Naturalist*, April 1931, p. 102.
On flies, Castle Rising Carr, Norfolk, August 24, 1930! Arncliffe Woods, Whitby, September 17, 1930!

**Cephalosporium dipterigenum* Petch in *Naturalist*, April 1931, p. 102.
On fly, Mulgrave Woods, Whitby, September 16, 1930!

**Aspergillus depauperatus* Petch in *Trans. Brit. Mycol. Soc.* XVI (1931), 244.
On *Lepidosaphes ulmi* on Hawthorn, Hunstanton, February 1921!

**Metarrhizium Anisopliae* (Metsch.) Sor.
On larva of *Sitones flavescens*, Swordale, Evanton, Ross-shire, coll. D. J. Jackson (E. M. Wakefield)!

**Beauveria Bassiana* (Bals.) Vuill. in *Bull. Soc. Bot. France*, LIX (1912), 40; *Botrytis Bassiana* Bals. in *Bibl. Ital.* LXXIX (1835), 125.
On insects of all kinds, among moss or dead leaves, or under bark or logs, etc.
On pupae, specimen in Herb. Kew., *ex* Herb. Cooke, dated 1885, *sub Isaria crassa*! Black Hills, North Wootton, March 23, 1930! West Briggs, near King's Lynn, September 2, 1930! Hagg Wood, near Burnsall, September 15, 1931!
On larva, Hagg Wood, near Burnsall, September 15, 1931!
On spiders, North Wootton, May 14, 1930! Holt House Wood, near King's Lynn, August 12, 1930! North Wootton, December 21, 1930!
On a fly, Holt House Wood, August 17, 1931! On a Tipulid, North Wootton, October 24, 1931!
On a Coccinellid, Bishop's Wood (Herb. Kew.)! ditto, Barton Mills, August 21, 1930 (Miss Brett)! On *Sitones lineatus*, Swordale, May 1919 (Miss D. J.

Jackson)! On *Hylastes ater*, Bagshot, March 24, 1922! On a weevil, Beck Hole, September 19, 1930! ditto, Shouldham Warren, April 17, 1931! On a weevil (*Otiorrhynchus sulcatus*), Seale Hayne, November 1930 (A. Beaumont)!

On an ant, Hagg Wood, near Burnsall, September 15, 1931!

On *Lecanium* sp., Cheddar, August 1926 (E. E. Green)!

**Beauveria densa* (Link) Vuill. in *Bull. Soc. Bot. France*, LIX (1912), 40; *Sporotrichum densum* Link, Obs. 1, 12 (1809).

Specimen, September 1837, Ch. Forest, C.B., on ? (in Herb. B.M.)! On a Pentatomid, Isle of Purbeck, January 1887, E. R. Bankes (in Herb. B.M.)!

On a spider, a weevil, and a coccinellid, Black Hills, North Wootton, March 30, 1930! On a beetle, Holt House Wood, April 3, 1930! On a weevil, North Wootton, December, 21, 1930! On an ant, Burnsall, September 12, 1931! On a spider, Hagg Wood, near Burnsall, September 15, 1931!

DEMATIACEAE

Cladosporium Aphidis Thuem. in *Oester. Bot. Zeitschr.* XXVII (1877), 12.

On *Macrosiphum dirhodum* Walker on Rose, Balconie, Evanton, Ross, coll. D. J. Jackson, October 1917 (A. D. Cotton, *Trans. Brit. Mycol. Soc.* VI (1919), 203).

On aphis, on Birch, Shouldham Warren, June 17, 1930! ditto, on Beech and Oak, Houghton Woods, Yorks., July 5, 1930! ditto, on Sycamore, Bishop's Wood, near Selby, July 11, 1931!

On flies, Leziate Fen, June 20, 1930! Hornsea, July 7, 1930! Castle Rising Carr, August 24, 1930! North Wootton, August 26, 1931, September 8, 1931! Holt House Wood, August 17, 1931, October 17, 1931! Bolton Woods, September 14, 1931!

Examples on flies usually follow an attack of *Empusa* or *Entomophthora*. It appears probable that *Cladosporium Aphidis* is merely saprophytic, and that, as has been claimed, it is *Cladosporium herbarum*.

STILBACEAE

**Stilbella Kervillei* (Quélet) Lingels. in *Ber. Deutsch. Bot. Gesellsch.* XXXIX (1921), 149; *Stilbum Kervillei* Quélet in Gadeau de Kerville, *Bull. Soc. Amis. d. Sc. Nat. Rouen*, 1884.

On flies, Cresswell Caves, Derbyshire, 1923 (F. A. Mason, *Journ. Bot.* August 1931, p. 205)!

Isaria farinosa (Holms.) Fries, *Syst. Myc.* III, 271 (1832); *Clavaria farinosa* (Holms.) Dickson, *Plant. Crypt. Brit.* 2, 25 (1790); *Ramaria farinosa* Holms. in *Nova Act. Dan.* I, 299, fig. 6 (1781); Sowerby, Pl. 308; *Isaria farinosa* Fr., Berkeley, *Eng. Flora*, V, pt. 2, 328 (1836), and *Outlines*, p. 338 (1860); Cooke, *Handbook*, pp. 548, 770, fig. 368 (1871), and *Vegetable Wasps, etc.* p. 178, fig. 36 (1892); Massee, *Brit. Fungus Flora*, III, 447, fig. 31, p. 397 (1893).

Specimens which appear to be morphologically indistinguishable from *Isaria farinosa* have been found on Coleoptera, Hymenoptera, Arachnida, Diptera and Aphidae (cf. Petch in *Naturalist*, April 1932, p. 107).

Spicaria (*Isaria*) *Swantonii* (A. L. Sm.) Petch in *Naturalist*, April 1931, p. 103; *Coremium Swantonii* A. L. Sm. in *Trans. Brit. Mycol. Soc.* VI (1919), 156.

On Hymenoptera. On a dead wasp (*V. sylvestris*), Blackdown, Haslemere, Surrey, coll. E. W. Swanton (A. L. Sm.). On bees, "found under stones," Caermarthen, February 7, 1878 (Herb. B.M.) !, and April 22, 1876, without locality (Herb. B.M.)! On bees and bee pupae, Mulgrave Woods and Beck Hole, September 1930! On cocoon of sawfly (*Lygaeonematus erichsoni*), Farnham Royal, November 1931 (J. C. Robbins)!

**Isaria leprosa* Fries, *Syst. Myc.* III, 272 (1832).

On lepidopterous pupae, Mulgrave Woods, Yorks., September 1930!

**Isaria dubia* Delacr. in *Bull. Soc. Myc. France*, IX (1893), 264, Pl. XI, fig. 1.

On larvae of *Hepialus* at the roots of grasses, Barr Beacon near Walsall, Staffs., April 1931 (E. W. Jones)!

**Hymenostilbe muscaria* Petch in *Naturalist*, April 1931, p. 101.

On flies and fly pupae.

Arncliffe Woods, Whitby, September 17, 1930! Malyan Spout, Goathland, September 19, 1930! Holt House Wood, near King's Lynn, August 17, 1931! Bolton Woods, Yorks., September 14, 1931!

Hymenostilbe arachnophila (Ditm.) Petch in *Naturalist*, August 1931, p. 249; *Isaria arachnophila* Ditmar in Sturm, *Deutschl. Flora*, Abt. III, Bd. 1, 111, Pl. 55 (1817).

On spiders, usually on the under surface of leaves.

Southampton, 1916 (J. F. Rayner)! West Briggs, near King's Lynn, August 21, 1931! Bolton Woods, Yorks., September 14, 1931! Burnsall, September 16, 1931!

This species has been confused in Europe with *Gibellula aranearum* (Schw.) Syd. In the absence of specimens it is not possible to say to which species the following records of "*Isaria arachnophila* Ditm." refer,—Tay, J. Stevenson, *Mycologia Scotica*, p. 263 (1879); Clyde, Essachossan, J. W. H. Traill in Fungi of Inverary, *Trans. Cryptog. Soc. Scotland*, 1888; Church Fenton, Ingrow, Elland Park and Masham, *Fungus Flora Yorks.*, p. 345 (1905); Sandsend, September 1913 (Y. N. U.); Forres, B.M.S. Foray, 1912; Swansea, B.M.S. Foray, 1915; Keswick, B.M.S. Foray, 1922. Berkeley's record, Collyweston, Northants (1838), appears, from the specimen and description, to have been a mistake.

[*Hymenostilbe Sphingum* (Schw.) Petch; *Isaria Sphingum* Schw., *Syn. Fung. Carol. Super.* p. 126 (1822).

Berkeley and Broome's record of *Isaria Sphingum*, on pupae of diptera, Kincardineshire (Taylor), *Ann. Mag. Nat. Hist.* Ser. 5, 1 (1878), 27 is an error.]

**Akanthomyces aculeata* Lebert in *Zeitschr. f. wiss. Zool.* IX (1858), 448, Pl. 17, figs. 13–17.

On moths.

White Hill, Auchter House, Dundee, November 1847 (G. Lawson)! Moffat, Dumfries, August 1861 (W. Carruthers)! St Mary's Isle, Kirkcudbright, no date (Lady Katherine Douglas)! New Forest, November 14, 1898 (G. Crawshay)! New Forest, no date (Mr Bond)! Without locality or date (Mr Bond)! Dartford Heath, no date (Mr Lynch)! Devon, no date (Miss Stawel)! Isle of Purbeck, March 3, 1886! July 22, 1887! October 21, 1889! (E. R. Bankes). Wareham, December 18, 1880 (O. C. Farrer)!; December 23, 1903 (E. R. Bankes)!

The first of these specimens is in Herb. Kew., the remainder in Herb. British Museum. The last five specimens are on *Hepialus humuli* on Gorse.

**Gibellula aranearum* (Schw.) Syd., Petch, *Naturalist*, August 1931, p. 249; *Isaria aranearum* Schw., *Syn. Fung. Carol. Super.* p. 126 (1822); *Gibellula pulchra* (Sacc.) Cav. in *Atti d. Ist. Bot. Univ. Pavia*, Ser. 2, III, 347 (1894); *Gibellula aranearum* Syd. in *Engler's Bot. Jahrb.* LVII (1922), 321.

On spiders attached to grasses or to the underside of leaves.

Worcester, B.M.S. Foray, September 1921 (E. M. Wakefield)! Camberley, August 18, 1926 (E. E. Green)! Cheddar, August 1926 (E. E. Green)! Chippenham Fen, August 1926 (C. P. Petch)! Freshwater, Isle of Wight, July 25, 1927 (E. E. Green)! Holt House Wood, near King's Lynn, October 17, 1931!

Hirsutella Eleutheratorum (Nees) Petch in *Naturalist*, February 1932, p. 46; *Isaria Eleutheratorum* Nees, *System d. Pilze*, p. 85 (1817).

On beetles.

Gray, *Natural Arrangement of British Plants*, I, 563 (1821), without locality. Aberdeen, in winter, J. W. H. Traill, *Report for* 1887 *on the Fungi of the East of Scotland*. North of England (A. W. Bartlett). Cambridge, January 1932 (Dr T. Harris)!

**Hirsutella subulata* Petch in *Naturalist*, February 1932, p. 48 ; *Isaria floccosa* B. & Br. in *Ann. Mag. Nat. Hist.* Ser. 5, VII, 130 (1881).

On a caterpillar, Milton, Northants. (J. Henderson), without date, in Herb. Kew.!

**Hirsutella acridiorum* Petch, n.sp.

On a grasshopper, Hagg Wood, Burnsall, September 15, 1931 ! On the only specimen found, the fungus does not form a clava, but a uniform covering of mycelium on which the phialides are borne laterally, as in *Hirsutella versicolor* and *Hirsutella floccosa*.

Hyphis crassis, 3 μ diam., regularibus, insectum membrana continua tenui cinerea vel pallide brunnea obducentibus; clavis nullis; phialidibus sessilibus, 16–28 μ altis, 2–3 μ diam., basi conicis vel leniter ampullaceis, media altitudine in sterigma tenue, 1 μ diam., abeuntibus, aliquando duo sterigmata ferentibus; conidiis ovalibus, inequilateralibus vel subcymbiformibus, apiculatis, 5–6 × 3–4 μ, vel obpyriformibus, 7 × 2 μ.

Syngliocladium nov.gen.

Synnemata filiformia ex hyphis parallelis coalitis composita, conidiophoros *Gliocladii* undique ferentia.

**Syngliocladium aranearum* Petch, n.sp.

On a spider among dead leaves, St Leonard's Forest, Horsham, May 31, 1931 !

Clavis gracilibus numerosis decumbentibus flexuosis simplicibus vel apicem versus furcatis, usque 5 mm. longis, 0·1 mm. diam., teretibus, albis, farinaceis, floccos nodulosos minutos undique ferentibus, apice obtuso vel sub-acuto; conidiophoris infra sparsis supra confertis, 25–33 μ altis, plerumque ramulis tri-ordinatis, globulos conidiorum primum globosos 18 μ diam. dein confluentes suffulcientibus; phialidibus anguste ampullaceis, 10–12 × 1·5 μ; conidiis hyalinis continuis ovalibus vel oblongo-ovalibus, 1·5–2·5 × 1 μ, vel globosis, 1 μ diam.

TUBERCULARIACEAE

Microcera coccophila Desm. in *Ann. Sci. Nat.* Ser. 3, X (1848), 359; Cooke, *Handbook*, p. 556, fig. 228 (1871), and *Vegetable Wasps, etc.* p. 322, Pl. 2, fig. 22 (1892); Massee, *Brit. Fungus Flora*, III, 486 (1893); Petch, *Trans. Brit. Mycol. Soc.* VII (1921), 115–124, Pl. III, fig. 8; *Atractium flammeum* Berk. & Rav., Berkeley, *Outlines*, p. 340 (1860); Cooke, *Handbook*, p. 555, fig. 227 (1871); Massee, *Brit. Fungus Flora*, III, 452, fig. 33, p. 397 (1893).

In Britain, usually on *Chionaspis salicis* on Willow.

Penzance (Ralfs)!; December 1869 (C. E. B.)!; Cooke, Fungi Brit. Exsicc., No. 350, and Fungi Brit. Exsicc. Ed. II, No. 354! Trengwainton, Penzance, December 1869! Ryde, Isle of Wight (A. B.)!

Recorded by Massee in *Fungus Flora of Yorkshire*, p. 345 (1905), for Scarborough, but probably in error.

Examination of the specimen shows that the record of this species for Woolwich in *Trans. Brit. Mycol. Soc.* VI (1920), 374, is incorrect.

Species dubiae

Xylaria apum Gray, *Natural Arrangement of British Plants*, I, 511 (1821).

"Stipes shortish, thick, tuberous at bottom; club granulated, chocolate-brown."

"On the pupae of bees."

"Stipes an inch long; head a quarter as long, one or two from the same base."

Isaria sphecophila Ditm.

A specimen on a bee, recorded and figured by Worthington G. Smith in *Gardeners' Chronicle*, n.s. XVII (1882), 378, bears some resemblance to this

species. Smith stated (*loc. cit.*) "The bee was caught in this country as it was inconveniently flying with its burden," and in a letter to Cooke, now preserved in Herb. Kew., he wrote "Michael Terry caught the wasp (*sic*) alive, I believe near Slough. He had it at the fungus exhibition at South Kensington in 1871 and gave the insect to me. I still have it in spirit" (dated 8. 12. 84).

Cooke reproduced the figure in his account of *Cordyceps sphecocephala* (*Vegetable Wasps, etc.* p. 50, fig. 12), but inverted it.

Fungi parasitic on entomogenous fungi

Melanospora parasitica Tul., *Selecta Fung. Carp.* III, 10, Pl. III, figs. 11–14.

On immature *Cordyceps militaris*, North Wootton, September 1880 (Plowright, *Grevillea*, X, 71). On *Isaria farinosa*, West Briggs, near King's Lynn, September 10, 1931!

Sporotrichum Isariae Petch in *Naturalist*, April 1931, p. 102.

On *Isaria farinosa*. Black Hills, North Wootton, March 1930! Mulgrave Woods, Yorks., September 1930! St Leonard's Forest, Horsham, May 31, 1931!

TUBER ZEYLANICUM B. & BR. AND *SCLEROTIUM ROLFSII* SACC.

By MALCOLM PARK, *Mycologist*
Department of Agriculture, Ceylon

(With Plate IX)

Some confusion has arisen regarding the nomenclature of *Tuber zeylanicum* B. & Br., *Sclerotium zeylanicum* (B. & Br.) Petch, *Sclerocystis coremioides* B. & Br., and *Sclerotium Rolfsii* Sacc., and this note is written in order to clarify the situation.

Tuber zeylanicum B. & Br.

This was described by Berkeley and Broome (1) in No. 975 of the *Fungi of Ceylon* as "Minutum, luteum rugosum, centro affixum (No. 1013). On a piece of dead stick. Peradeniya, Dec. Only a single specimen found, 1½ lines across, unfortunately without perfect fruit. No. 1093 is in the same condition." The description is repeated in Saccardo (2), but the diameter is given as 19 mm., apparently an error in transcription.

The type (Thwaites No. 1013) was divided and one part is in Herb. Kew., the other in Herb. Perad. A piece of the Herb. Perad. specimen was microtomed in 1926 and the sections, a photograph of one of which is reproduced (Pl. IX, fig. 1), show that the fungus resembles a *Tuber*. The specimen in Herb. Kew. was examined by Messrs S. F. Ashby and E. W. Mason of the Imperial Mycological Institute, Kew, and Ashby described it in a letter to the Mycologist, Ceylon, dated March 7th, 1927, thus: "It consists only of the half of a fruit body and answers to the description, being rather less than 4 mm. in diameter. It is orange externally and shows a labyrinthine internal structure as seen under a hand lens like one of the *Tuberaceae*." Mason made a rough sketch of the internal surface of the specimen, which has been copied and elaborated somewhat with the assistance of the sections in Herb. Perad. (Pl. IX, fig. 4).

Thwaites No. 1093. In Berkeley and Broome's description of *Tuber zeylanicum* quoted above, reference is made to No. 1093 as being "in the same condition," *i.e.* presumably, "unfortunately without perfect fruit." Ashby, in the letter cited above, says that "Thwaites No. 1093 is mounted on the same sheet as No. 1013 in Herb. Kew.; it was collected in January 1869. Under it is written *Tuber zeylanicum* in Cooke's handwriting. It is a sclerotium-like body about 9 mm. long and 4 mm. high with a homogeneous white interior and is attached to a decayed fibrous fragment of a grass. Cooke was evidently in error;

Berkeley did not suggest that it was the same thing as 1013." The specimen of Thwaites No. 1093 in Herb. Perad. is probably a portion of the specimen in Herb. Kew. A piece of this specimen was microtomed in 1926 and a photograph of one of the sections is reproduced (Pl. IX, fig. 2). It will be seen that the interior is homogeneous, with the inner loosely woven hyphae running generally in a radial direction. The structure is bounded by a thickened "epidermis." Lying just inside the periphery over part of the surface are subspherical cavities which appear to be immature perithecia. It appears then that Thwaites 1093 is the immature stroma of a Pyrenomycete. It may be definitely dissociated from *Tuber zeylanicum*.

Sclerocystis coremioides B. & Br.

Petch published in 1908(3) a re-description of *Sclerocystis coremioides* based on an examination of herbarium specimens at Peradeniya. In 1912(4) he withdrew this re-description subsequent to von Höhnel's work on the co-type at Kew which led to a re-examination of the Peradeniya specimens and to the discovery that Thwaites No. 155, *Sclerocystis coremioides*, was a mixture containing at least one sterile sclerotium, which had been examined previously. This explanation was repeated in Petch's discussion of *Tuber zeylanicum* in 1931(5).

The details above are given to lead up to the point that Petch linked the sclerotium from Thwaites No. 155, *Sclerocystis coremioides*, with *Tuber zeylanicum*, which he referred to as a sclerotium in 1908(3), in 1910(6) and in 1931(5). The subsequent confusion in nomenclature is due to this erroneous observation.

Sclerotium zeylanicum (B. & Br.) Petch.

In 1908(3) Petch stated that on the germination in damp sand of large sclerotia, assumed by him to be *Tuber zeylanicum*, small sclerotia developed similar to the one he examined from the mixed Thwaites No. 155, *Sclerocystis coremioides*, and linked the two under *Sclerotium zeylanicum* (B. & Br) Petch. The name was used in various reports, etc., *e.g.* in 1922(7), but "it was unfortunately overlooked that no formal correction had been published."(8) The fungus was finally re-described as *Sclerotium zeylanicum* (B. & Br.) Petch in 1923(8), the description then given being "Sclerotia produced on fleecy, white, superficial patches of mycelium overrunning plants or soil, white, then pale ochraceous, or pinkish, finally brown, glabrous, globose, about 1 mm. diameter, internally white, hard, compact." In this description the formation of large "sclerotia" is not mentioned. Ashby stated in the letter referred to above that "*Sclerotium zeylanicum* (B. & Br.) Petch is represented in Herb. Kew. by Petch 6472 on *Aristolochia leuconeura*. Peradeniya, 1922. The clustered sclerotia are 0·8–1 mm. in diameter, yellow to brown and have a white homogeneous interior; there are

Fig. 1

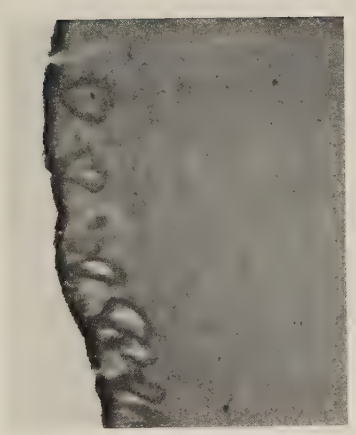

Fig. 2

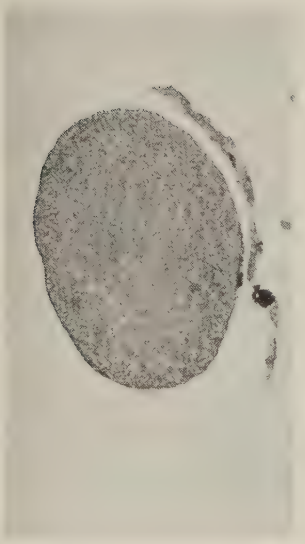

Fig. 3

Fig. 4

also strands of mycelium. This is the fungus we are calling *Sclerotium Rolfsii*."

Sclerotium Rolfsii Sacc.

This was described in 1911 by Saccardo (9) from specimens received from Florida. His description is "Superficiale, subrotundum v. horizontaliter, 0·5–0·8 mm. diam., facile secedens, levigatum, nitidulum, roseum, demum fulvescenti-brunneum, carnoso-firmulum, intus pallidum; cellulis e globose polyedricis 6–8μ diam. interdum sinuosis, subhyaline, ad peripheriam sclerotii brunneolis; fructificatione nulla observata." This fits admirably Petch 6472. Bertus in 1929 (10) pointed out that Petch's combination (*Sclerotium zeylanicum*) appeared to have been founded on *Tuber zeylanicum*, suggested that it was incorrect and adopted the name of *Sclerotium Rolfsii*. To this Petch replied in 1931 (5), pointing out that *Sclerotium zeylanicum* was, in fact, founded on *Tuber zeylanicum* and that it therefore had priority over *Sclerotium Rolfsii*.

It has been shown above that *Tuber zeylanicum* and *Sclerocystis coremioides* are separate fungi and distinct from *Sclerotium Rolfsii*. *Sclerotium zeylanicum* in consequence dates from its description in 1923 and, being identical with *Sclerotium Rolfsii*, the latter name has priority.

The writer wishes to acknowledge his indebtedness to Messrs S. F. Ashby and E. W. Mason of the Imperial Mycological Institute, Kew, for their notes on the relevant specimens in the Kew Herbarium.

EXPLANATION OF PLATE IX

Fig. 1. Section of Thwaites No. 1013, *Tuber zeylanicum*, in Herb. Perad. × 43.
Fig. 2. Section of Thwaites No. 1093, in Herb. Perad. × 43.
Fig. 3. Section of sclerotium of *Sclerotium Rolfsii* for comparison with Figs. 1 and 2. × 46.
Fig. 4. Elaborated sketch of cut surface of Thwaites No. 1013, *Tuber zeylanicum*, in Herb. Kew. × 23.

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(3) Petch, T. "A Preliminary Note on *Sclerocystis coremioides* B. & Br." *Ann. Bot.* xxii (1908), 116.
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OBSERVATIONS ON A NEW SPECIES OF *OLPIDIUM* OCCURRING IN THE ROOT HAIRS OF *AGROSTIS*

By KATHLEEN SAMPSON, M.Sc.

(With Plates X, XI and XII and 5 Text-figures)

Introduction

In October 1930 some seedlings of *Agrostis stolonifera* which had failed to make normal growth in the field were examined after staining with lacto-phenol cotton blue. Mycelia of varied form were present, including a fine non-septate type which occasionally carried spherical sporangia resembling *Pythium*. The method of decay was also suggestive of a *Pythium* rot, but the supposed pathogen was not isolated. The most striking feature of the preparations was the abundance of large, deeply stained bodies which are probably identical with the organism considered by Massee to be *Cladochytrium graminis* Büsgen*. These were present in all the decayed parts, including the coleoptile and the first leaves, and while attempting to identify them I discovered the resting spores of the species of *Olpidium* here described. The resting spores, which were contained in peculiar apical swellings of the root hairs, were slightly smaller than the *Cladochytrium* cysts and had somewhat pointed instead of rounded ends, but they resembled them in the possession of a thick wall which stained blue.

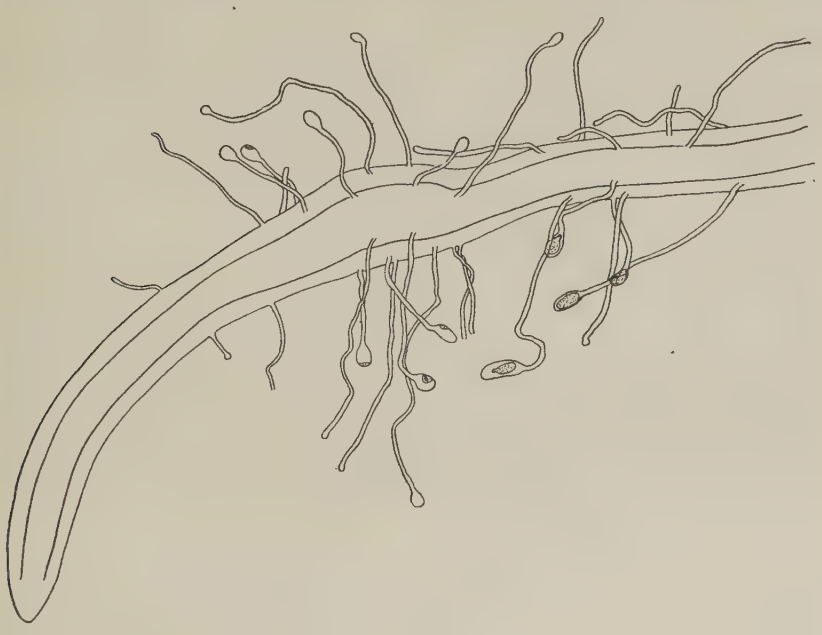
In order to obtain more material, the soil in which the seedlings had grown was allowed to become nearly air-dry, mixed with sterilised garden soil in pots and sown with seed of *Agrostis* on November 24th, 1930. Seedlings were removed and examined from time to time, and on January 27th, 1931, another sowing was made in the same soil. The pots usually stood in the open cage, but at intervals they were left in the laboratory for several days together. The soil was kept in a somewhat waterlogged condition by permitting little drainage. The "damping off" symptoms of the original material failed to reappear, but the cysts of *C. graminis* were often found. A few root hairs on nearly all the seedlings examined in the early months of the year possessed the typical swellings shown in Text-fig. 1. They provided material for studying the development of an unrecorded species of *Olpidium*.

Three important stages in the life cycle have not yet been observed,

* G. Massee. A new grass parasite, *Cladochytrium graminis* Büsgen. *Journ. Board Agric.* xx (1913–4), 701. The generic position of this organism is doubtful.

namely, the actual entrance of the zoospore into the epidermal cell, the fusion of gametes and the germination of the mature resting spore, but allowing for these gaps, there is strong reason to think that the species possesses a life cycle similar in its essential features to that of the best known species *O. Viciae*, which has been so fully described by Kusano (8).

A number of the observations have been made on living material. The roots were carefully washed free from soil and mounted at once in water. Preparations were sometimes irrigated with Coon's solution and kept in moist chambers for some days. Such "cultures" were



Text-fig. 1. Infected root showing the parasite in the swollen tips of root hairs. × 110.

by no means free from bacteria or other organisms, but the root hairs remained sufficiently healthy to permit of the development of the parasite from very young stages to the nearly mature zoosporangium or resting spore. This development was followed on several occasions by watching the changes in a single hair in order to prove that the stages described belong to a single organism.

Methylene blue, osmic acid and other reagents were helpful in the study of the earliest stages, while the material which gave the best cytological details was that killed in Navashin's fixative and stained with Haidenhain's haematoxylin.

The zoosporangium

The earliest stage of the parasite which I could discover is shown in Pl. X, fig. 1, in which a single amoeba is present in the root hair. On several occasions I was able to watch zoospores which had come to rest on the epidermis or on young root hairs, but I never saw one actually penetrate the wall. In *O. Viciae*, which attacks aerial parts of the plant, the zoospore encysts before entering and the wall of the cyst may be seen as a minute globule attached to the wall of an invaded cell. If the same process occurred here one would expect at least some of the infected hairs to carry empty cysts, but I failed to find any sign of these in the many hundreds of hairs examined. I am inclined to believe that the zoospore of this species enters without encystment as in *O. radicale* Schwartz and Cook (11).

The zoospores (Pl. X, figs. 3–5) possess either one or two refractive granules, which form a conspicuous feature of the parasite in the root hair (Pl. X, figs. 6–10). Unstained, living material gives the impression of a plasmodium formed by the coalescence of several amoebae, but staining *in vitro* with methylene blue makes it possible to distinguish the minute individual parasites clustered round the nucleus of the host. The cytoplasm of the amoeba takes up at first a slightly deeper tint than the host nucleus, while the granules remain colourless and make a clear contrast. These granules become nearly black with osmic acid solution, while the nucleus of the hair stains more slowly, and this reagent also serves to distinguish the individual amoebae from the contents of the host cell. With cotton blue it is usually impossible to trace the limits of the parasite until the young thalli have formed a membrane.

In living material the protoplasmic mass, consisting of nucleus and clustered amoebae, changes in shape and position during the first twenty-four hours after invasion (Pl. X, figs. 6–10). Rapid circulatory movements can be seen in the cytoplasm of the hair, accompanied by changes in the size and shape of the vacuoles. These movements are considered to be responsible for the apparent wanderings of the parasite in the hair. A careful study of the youngest hairs suggests that zoospores invade the epidermal cells not far from the root apex and that they are carried by cytoplasmic streaming to the tip of the hair during the course of its development. This sometimes fails to happen and the parasite then develops either in the epidermal cell or in the narrow space provided by a hair of normal width (Text-figs. 2–4), but in the material examined such conditions were rare. The normal habitat of the organism seems to be the swollen tip of a root hair. Direct infection of a root hair probably also takes place.

Roots kept in a culture solution between slide and coverslip remain alive for some days and permit the development of the parasite to proceed. Figs. 11–13 in Plate X were drawn from a root which carried seventeen hairs at a similar stage of development. In some hairs further growth was arrested, but in a number of them the parasite developed normally and sixteen hours later showed relatively large and quite distinct thalli with clearly marked membranes. Further development proceeded slowly, but a week later a few thalli

Text-fig. 2. Piliferous layer of root in surface view showing young thalli in epidermal cells. ×650.

Text-fig. 3. Zoosporangium in epidermal cells. ×640.

Text-fig. 4. Immature resting spores in unswollen root hair. ×640.

were found with exit tubes and nearly mature zoospores. Discharge of the zoospores was not obtained under these artificial "culture" conditions.

Mature zoosporangia which have developed normally in roots in soil are somewhat oblong, thin walled, and vary in size from 30–68 × 10–23μ. Each possesses one to four exit tubes. The range in size and shape of fully developed zoosporangia is illustrated in Text-fig. 5. The shape is partly governed by that of the root hairs and the pressure on it of soil particles. The size is probably correlated with the amount of available nutriment, which must depend to some

extent on the number of individuals in the cell, but relatively small zoosporangia may occur alone in large apical swellings. The exit tubes are usually very short, piercing the wall at the nearest point (Pl. XI, fig. 18). Sometimes they attain a considerable length before penetrating the cell wall (Text-fig. 5). As many as ten separate thalli may be found in one hair, and observations on the sequence of development support the view that normally each has its origin in a single zoospore which maintains its individuality and forms one zoosporangium. The somewhat peculiar shape of the largest thallus in Text-fig. 5 suggests, however, the possibility that a zoosporangium may sometimes have a multiple origin in the coalescence of very young thalli at a stage prior to the formation of a firm membrane.

Text-fig. 5. Zoosporangia showing range in size, shape and number of exit tubes. ×650.

The escape of the zoospores has been witnessed several times. It usually takes place not long after the roots have been washed and mounted. The first zoospores escape with a rush and quickly disperse, since they are not encased in any mucilage (Pl. X, fig. 3). A few come out alone, or in small numbers, and these are sometimes followed by a second large mass. Occasionally nearly ripe zoosporangia discharged zoospores which appeared to be normal except for the absence of cilia (Pl. X, fig. 5).

Mature zoospores measure approximately 2·2μ in diameter. When they first emerge the body is nearly spherical with a clear vacuole and one or two highly refractive granules. While swimming freely the long posterior cilium trails behind. If it gets entangled the spore tugs to release itself and in so doing the body of the zoospore presents a variety of irregular shapes. Zoospores were never obtained in great

numbers and I was not able to determine if they possess two periods of activity such as Kusano demonstrated for *O. Viciae*.

The resting spore

Typical resting spores which have been stained with cotton blue and mounted in lacto-phenol are shown in Pl. XI, figs. 23, 24. They are broadly elliptic or ovoid and usually measure 20–30 × 9–17μ. When they have developed in unswollen hairs, a rare occurrence, they are long and narrow (Text-fig. 4). The coat is thick, hyaline and perfectly smooth. In the stained specimens figured the coat measures 2·5μ at the side and 4 to 5μ at the ends where it is usually thickest. In a large and relatively mature spore (Pl. XI, fig. 23) it is clear that the coat possesses three well-marked zones which have been found to differ in their reactions to reagents (see p. 189). The middle zone stains with cotton blue and sometimes contains flecks of deep blue, but the inner and outer layers do not take up the stain.

The original plants revealed the resting spore stage only and these were few, but cultures of *Agrostis* studied at Aberystwyth showed the following sequence of stages. Seedlings from a sowing made on November 24th, 1930, contained in December and January only zoosporangia. In February and March 1931 zoosporangia and resting spores were found in nearly equal numbers, while in May the temporary sporangia were relatively infrequent and resting spores abundant. During the period of rapid root development which warmer weather encouraged, it became increasingly difficult to find any stage of the parasite. The facts seem to suggest a seasonal periodicity in the organism, but it is equally probable that the relative proportion of temporary and resting sporangia at any season is influenced by the growth rate and other conditions in the host plant and only indirectly by the season*.

Early stages in the development of resting spores have been followed in living root hairs. In the "cultures" previously described the change from minute amoebae to nearly mature individuals having a fairly thick coat occupied from seven to ten days. The time would probably be less if the hairs were left undisturbed in the soil. The value of the observations lies in the proof they give that the zoosporangia and resting spores belong to the same organism. Thalli which are destined to form resting spores can be recognised at an early stage in living material by their denser contents and by the striated appearance round the edge of the thallus which precedes the appearance of a thick wall (Pl. XI, fig. 22). Some root hairs kept under observation developed both temporary sporangia and resting

* These points could not be studied since the organism has not been seen since July 1931, although fresh sowings of *Agrostis* have been made twice on the same soil.

spores (Pl. X, figs. 11–13), while in others only one type was formed (Pl. XI, figs. 15–17).

Unfortunately no signs were obtained of the germination of the resting spores. It is probable that complete development is attained only after the spores have been liberated by the disintegration of the root hairs, and I failed to find free spores either in the soil or attached to old roots. This is not surprising in view of their minute size and relative scarcity.

The cytology of the genus *Olpidium* has been studied in few species. The most complete account is that given for *O. Viciae* by Kusano, who successfully demonstrated the fact that the resting spore results from a fusion of two zoospores, the nuclei of which remain associated but distinct during the growth of the thallus and fuse only in the mature resting spore shortly before germination takes place (8). Having failed to observe the fusion of gametes in the species under discussion, it seemed advisable to discover if possible whether the resting spores were binucleate. This fact has been definitely established.

Preparations made by staining the complete root gave clear evidence of the existence in certain thalli of two relatively large bodies with a marked affinity for nuclear stains (Pl. XII, fig. 25). It was finally proved by microtome sections, cut 6μ in thickness, and stained with Haidenhain's haematoxylin, that these bodies were nuclei surrounded by a substance which also retained the stain. Only young zygotes could be sectioned since no fixative was found which would penetrate the coat of the resting spore once the inner wall had been laid down.

In the microtome sections of root hairs the thalli are clearly of two types. Some contain a number of relatively small nuclei and no doubt represent young zoosporangia (Pl. XII, figs. 27, 28). Nuclear division was not observed. Other thalli are distinctly binucleate and these agree in size and shape with those which in living material ultimately become invested with a thick wall (Pl. XII, fig. 26). A striking feature of the binucleate thalli is the stellate form of the deeply staining substance in the cytoplasm round each nucleus, which may be taken to indicate that extrusion of chromatin material has taken place. This substance, coupled with the fact that the nuclei themselves are large relative to those of the zoosporangia, makes the pair of nuclei in young resting spores clearly visible even in material stained in bulk. Kusano has recorded the extrusion of nuclear material from the nuclei of young resting spores in *O. Viciae*, and a similar phenomenon was described by Curtis in *Synchytrium endobioticum* (5), and by Schwartz and Cook in *O. radicale* (11).

The binucleate condition, with its exact parallel in *O. Viciae*, indicates that the resting spore is a zygote formed by the copulation of zoospores. The fusion was not witnessed, but the earliest stages studied

gave no evidence of copulation subsequent to invasion of the host cell, and I am inclined to think that union takes place in the film of soil water before the parasite invades the root.

The coat of the resting spore

In all the material available for study, the thick coat which characterises the resting spore was colourless. The oldest spores are found to possess a coat with three distinct zones; a smooth hyaline exospore, a thin colourless endospore and a space between of variable diameter, which absorbs the cotton blue stain and contains, not infrequently, particles of a substance which stains deeply with this reagent (Pl. XI, fig. 23).

Tests were made under the microscope, with a few stains and other reagents, on fresh resting spores at different stages of development. The number of tests on mature spores was strictly limited by the scarcity of material, but the results, which are tabulated below, give consistent evidence of a difference in the reactions of the inner and outer membranes.

Reagent	Action on wall of root hair	Action on exospore	Action on endospore
Chlorzinc-iodine	Blue-grey	Negative (Spore as a whole bright yellow)	Negative
Azo blue	Blue	Faint blue	Not stained
Congo red	Red	Red	,,
Methylene blue	Not stained	Not stained	Violet-blue
20 per cent. potash solution	Swollen	Swollen	Negative
Zinc-chloride in hydrochloric acid*	Dissolved in a few hours	Dissolved in a few hours	Negative
Concentrated sulphuric acid	Dissolved rapidly	Dissolved rapidly	Not affected for several days

The spores shown in Pl. XII, fig. 31, were present in a root which had been mounted in 20 per cent. potash and left exposed on the bench for twelve hours. The exospore became swollen and then wrinkled, giving the spore a stellate form in optical section. When preparations were irrigated with a solution of zinc chloride in hydrochloric acid, or with concentrated sulphuric acid, this stellate wrinkling was instantaneous, followed by the rapid expansion of the exospore and finally by its solution. In these reagents the exospore proved to be nearly as soluble as the cellulose walls of the root hairs, but the endospore was extremely resistant, remaining unaffected for days. Pl. XII, fig. 29, shows three resting spores mounted in the zinc reagent; fig. 30 shows one of the spores three hours later with only the endospore and the globular contents left. In this condition it remained for several days.

* See P. Haas and T. G. Hill, *An introduction to the chemistry of plant products*, vol. 1, 4th ed. p. 209 (1928).

It is evident that the exospore and endospore of the resting spore are not identical in chemical composition. The reactions of the former suggest affinities with cellulose, but I was not able to obtain the required colour with chlorzinc iodine. It is possible that this was masked by the bright yellow colour assumed by the contents of the spore. The extreme resistance of the endospore to the solvents employed suggests that it is probably of pectin origin (7).

Kusano (8) described in *O. Viciae* a yellow, brittle exospore, and a thicker hyaline endospore, which he was able to separate by the use of chloral hydrate; both walls failed to give the cellulose reactions. In *O. Trifolii* he (9) described spores which, after treatment with caustic soda, gave the appearance of a wall with three zones. He suggests that a three-layered wall may be characteristic of resting sporangia in the genus *Olpidium*. Němec (10) states that cysts of *O. Salicorniae* possess a wall having several layers.

The coat of the resting spore in the present species is certainly not a single uniform layer. It may best be described as consisting of two smooth, hyaline walls which have a similar appearance, but differ in chemical composition. Between them is a space of variable thickness which probably contains some cytoplasm, since it retains a certain amount of the cotton blue stain. The tests were not sufficiently extensive to determine the chemical composition of the three layers, but they point to the possibility that the exospore has affinities with cellulose while the endospore resembles the pectic substances.

Discussion

Excluding *O. Lemnae* Fisch. and those parasitic on the pollen of Phanerogams the following is a list of the species of *Olpidium* which have been recorded on higher plants.

On Leaves.

(1) *O. Viciae* Kusano on *Vicia unijuga* in Japan.

(2) *O. Trifolii* Schroet. on *Trifolium repens* in Silesia and in Japan. This species differs from (1) only in the smaller size of the temporary and resting sporangia and in its infection capacity (9).

(3) *O. simulans* de Bary and Wor. On *Taraxacum* spp. According to Kusano this species is similar to *O. Trifolii*, but it occurs on a host which the *Trifolium* species will not infect (8).

On Roots.

(4) *O. Brassicae* (Wor.) Dang. (= *Asterocystis radicis* de Wildem.) occurs on the roots of *Brassica* sp., flax, tomato, tobacco (3), various Gramineae and other plants. Its chief characteristic is the stellate form of the resting spores. Although so frequently recorded it is not so fully known as *O. Viciae*.

(5) *O. radicicolum* de Wildem. (= *O. Borzii* de Wildem.) on *Brassica* sp. This species has been separated from *O. Brassicae* on the basis of length of exit tubes and on the sculpturing of the wall of the cyst (1). I am doubtful if the distinction is valid, since the length of the exit tube is usually governed by the distance between the zoosporangium and the epidermal wall of the host, and the supposed difference in the wall has never been clearly defined or figured.

(6) *O. Salicorniae* Němec in the hypodermis of the roots of *Salicornia* sp (10). The zoosporangia are said to agree closely with those of *O. Brassicae* except for the almost complete absence of exit tubes. The cysts however have a smooth wall composed of several layers and therefore differ markedly from those of *O. Brassicae*.

(7) *O. radicale* Schwartz and Cook on the roots of *Veronica Beccabunga*, *Poa annua* and *Lepidium sativum* (11). This species presents some peculiar features, viz. the absence of exit tubes in the zoosporangia and the conjugation of the zoospores within the host cells. The resting spores are described as spherical, with a smooth wall.

The present species differs from those mentioned above in several important features. It inhabits the root hairs, causing definite hypertrophy, the zoosporangia vary considerably in shape but are on the whole oblong, and possess one to four exit tubes of variable length. Finally, the resting spores are definitely ovoid with a thick smooth coat. In its general life cycle, so far as it is known, this species resembles *O. Viciae*.

The genus *Pleotrachelus* was founded by Zopf on a species inhabiting *Pilobolus*, which possessed zoosporangia with many (up to thirty) long, slender exit canals (13). One species, *P. radicis* de Wildem., has been recorded on the roots of plants (12), but the zoosporangia possess a large number of exit tubes and show no resemblance to the present species on *Agrostis*.

The distinction between the genera *Olpidium* and *Pleotrachelus* has been found difficult to maintain, since the zoosporangia in well-known species of *Olpidium* vary in the number of exit tubes. Thus Kusano described zoosporangia in *O. Trifolii* which possessed as many as twelve exit tubes.

Rhizomyxa hypogaea Borzi (4) is said to cause occasional weak swellings of the root hairs, and *Agrostis alba* is among the hosts listed. It is probably a mixture of several organisms, but none of the figures given* shows much resemblance to any stage of the parasite under discussion. It seems best, therefore, to describe the parasite as a new species of *Olpidium*, and the following diagnosis is given.

* I have not been able to see the original paper by Borzi. Reproductions of his figures are given in Rabenhorst, *Krypt Flora*, vol. 1, Abt. 4, p. 67, and in Fitzpatrick, *The Lower Fungi: Phycomycetes*, p. 62.

Diagnosis: *Olpidium Agrostidis* nov.sp.

Zoosporangia oval to elongate, variable in size, measuring 30–68 × 10–23μ, solitary or aggregated, occurring in the root hairs of *Agrostis*. Exit tubes usually short, but varying in length according to the distance between the sporangium and the wall of the hair.

Zoospore colourless, more or less spherical, about 2·2μ in diameter, with one or two hyaline granules and a long posterior cilium. Zoospores discharged without mucilage. Resting spores solitary or aggregated, one to ten per root hair, occurring alone or mixed with the zoosporangia. Broadly elliptical or ovoid, 20–30 × 9–17μ, possessing a thick coat consisting of two smooth hyaline walls separated by a middle layer of variable diameter. Germination not observed. Invariably binucleate to a mature age, suggesting that their origin is by the copulation of zoospores.

On *Agrostis stolonifera* from Bingley, Yorkshire, causing apical swellings of the root hairs. Found rarely in the base of a hair or in other epidermal cells.

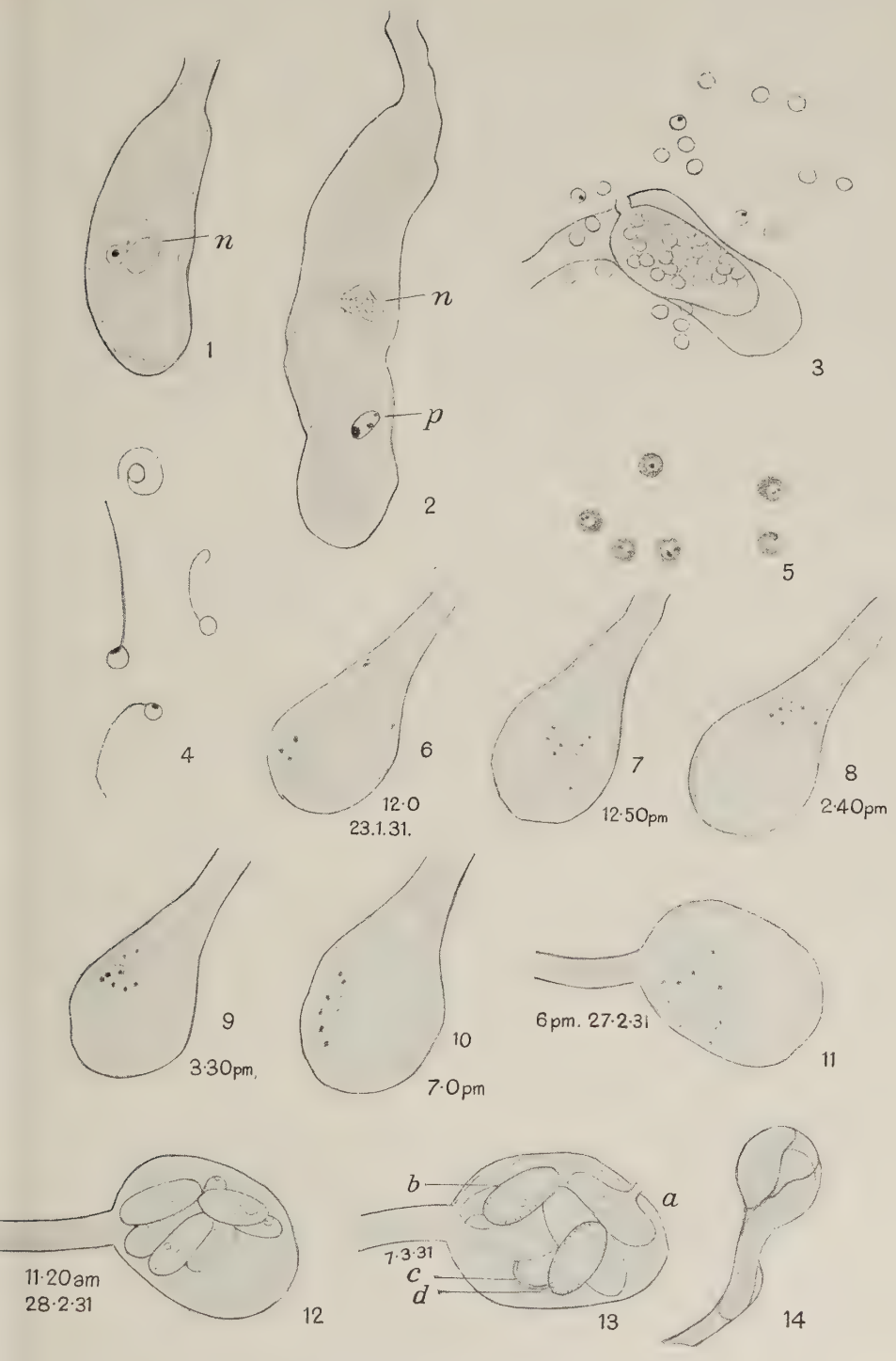
Olpidium Agrostidis nov.sp.

Zoosporangiis ovalibus elongatisve, 30–68 × 10–23μ, singulis aggregatisve, in Agrostidis radicellis endophyticis; zoosporis pallidis, ± globosis, c. 2·2μ diam., granulis hyalinis 1–2 et cilio posteriori longo praeditis; sine mucilagine expulsis; sporis perdurantibus singulis aggregatisve, in quoque rhizoideo 1–10 solis vel cum zoosporangiis intermixtis, ample ellipticis ovoideisve, 20–30 × 9–17μ, tunica crassa triplici praeditis, semper binucleatis; germinatione haud observata. In Agrostidi stolonifera radicellis tumores apicales efficiens.

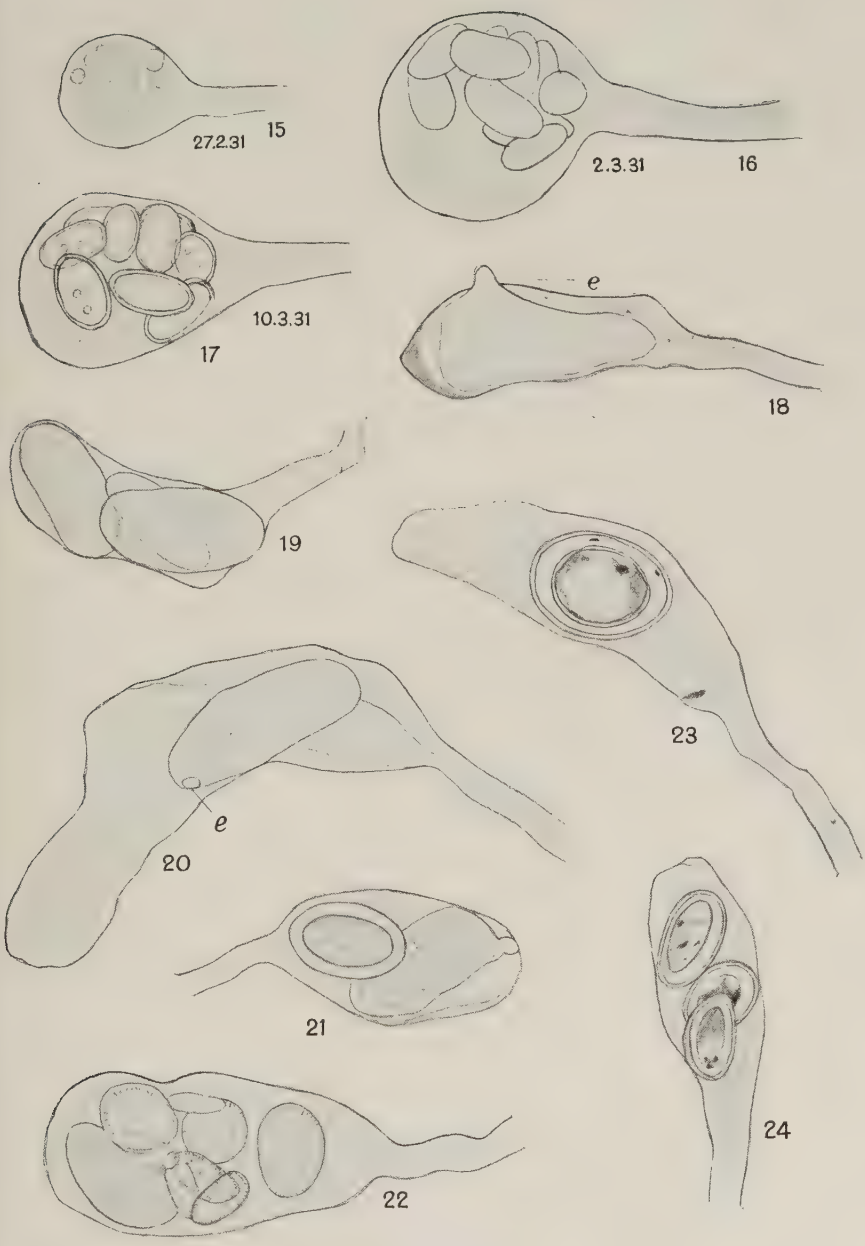
In conclusion I desire to thank Dr E. J. Butler, Mr J. Ramsbottom and Dr A. W. Bartlett for the loan of original papers, and Dr Vranek, of the Department of International Politics, Aberystwyth, for his kindness in translating papers written in Czech.

Thanks are also due to Miss G. M. Roseveare, Bureau of Plant Genetics, Aberystwyth, for valuable assistance in obtaining various foreign periodicals.

I am indebted to Mr R. B. Dawson, Director of the St Ives Research Station, Bingley, who provided the material of *Agrostis* upon which this *Olpidium* originally appeared, and to Prof. R. G. Stapledon for the use of facilities provided by the Welsh Plant Breeding Station.



Olpidium Agrostidis nov.sp.



Olpidium Agrostidis nov.sp.

EXPLANATION OF PLATES X, XI, XII

All the drawings, except Fig. 4, were made with a camera lucida.

Plate X

All the drawings were made from living material. Except where stated the magnification is × 675.

Fig. 1. Early stage of infection. Amoeba near nucleus of root hair.

Fig. 2. Later stage. In this case the parasite is separated by a vacuole from the nucleus of the hair. *n* = nucleus; *p* = parasite.

Fig. 3. Discharge of zoospores from zoosporangium.

Fig. 4. Living zoospores.

Fig. 5. Zoospores without cilia, discharged 27. ii. 31. × 750.

Figs. 6–10. Showing changes in the position of host nucleus and amoebae between 12 noon and 7 p.m. on 23. i. 31.

Fig. 11. Invaded root hair at 6 p.m. on 27. ii. 31. On one root seventeen hairs were in this condition at the same time.

Fig. 12. Seventeen hours later, distinct thalli with limiting membrane.

Fig. 13. Eight days later. (*a*) zoosporangium with exit tube; *b*, *c* and *d* show the initial stages of the thick coat characteristic of resting spores.

Fig. 14. Three young zoosporangia in a root hair.

Plate XI

All the drawings were made from living material except Figs. 20 and 24, which were made from material fixed in alcohol and stained with lacto-phenol cotton blue. Magnification in each case × 675.

Fig. 15. Hair packed with amoebae, 27. ii. 31.

Fig. 16. Same hair, 2. iii. 31, showing ten thalli with coarsely granular contents.

Fig. 17. Same hair, 10. iii. 31. All the thalli have formed a thick coat.

Figs. 18–20. Young zoosporangia. *e* = exit tube.

Fig. 21. Nearly ripe zoosporangium and a resting spore in the same hair.

Fig. 22. Zoosporangium; five immature resting spores and three thalli which have been arrested in growth.

Fig. 23. Resting spore stained with cotton blue showing exospore and endospore. Between these walls lie particles of material which have taken up the stain.

Fig. 24. Three resting spores stained with cotton blue.

Plate XII

Fig. 25. From a root stained *in toto* with Haidenhain's haematoxylin showing four immature resting spores each with two nuclei. × 1375.

Fig. 26. From a section of a root hair cut 6μ and stained with Haidenhain's haematoxylin, showing three young resting spores each of which is binucleate. The second nucleus of one spore lies in another section. The nuclei possess a large nucleolus and are surrounded by a stellate mass of deeply staining material in the cytoplasm. × 1375.

Figs. 27, 28. Sections of zoosporangia showing numerous nuclei. From the same preparations as Fig. 26. × 1375.

Fig. 29. Resting spores from a root mounted in a solution of zinc chloride in hydrochloric acid. The wall of the root hair has begun to dissolve. The exospore and the endospore are clearly visible while the contents of the spore have formed a sphere in the centre. × 675.

Fig. 30. One of the same spores a few hours later. The exospore has been dissolved but the endospore remains intact. × 675.

Fig. 31. Two resting spores mounted in 20 per cent. potash solution and left for twelve hours exposed to the air. The exospore is wrinkled. Spore *a* is seen in surface view; spore *b* in optical section. × 675.

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CERCOSPORA FABAE FAUTREY, ON FIELD BEANS

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(With Plate XIII)

I. Introduction

Cercospora Fabae Fautrey, causing spotting of the leaves of *Vicia Faba*, was observed at South Leigh, Oxfordshire, on January 15th, 1927. This is believed to be the first reported occurrence of this fungus in England. Apart from the original description by Fautrey in 1891 it has been reported only by Ferraris from Piedmont in 1910, Stevenson (7) as occurring in China, Russia, Italy, Bohemia and France, and in 1927 by Petri in Italy. Since 1927 it has been found widely distributed in England, occurring in Northamptonshire, Herefordshire, Warwickshire, Kent, Cambridgeshire, Berkshire, Gloucestershire, and other counties. The disease was discovered during observations on field beans for Chocolate Spot. The close resemblance between the Cercospora Spot, Chocolate Spot, and other Leaf Spot diseases led to a careful examination of them to determine the prevalence and distinguishing characteristics of each.

II. Symptoms

The disease has been noted in Oxfordshire during all seasons of the year, having been found on self-sown plants during late autumn, and on autumn-sown plants during January and throughout the period of active growth. During spring and early summer it often causes extensive spotting of the foliage over the greater part of the plants when conditions favourable for its spread, namely damp and warm weather, appear. The lower leaves are much more subject to attack than those nearer the top of the plant, the latter usually remaining free from the fungus. This is especially noticeable during the winter when infection is commonly confined to the lower leaves. If favourable conditions do not prevail when growth begins during spring, the disease ceases to spread and the plants grow rapidly away from it, so that the later formed foliage is healthy.

Any part of the lamina of the leaf may be attacked and occasionally lesions may occur on the stem. In the field the first sign of fresh infection takes the form of chocolate-coloured spots 1 mm. or so in diameter which appear on leaves already having one or more fully developed spots, or on foliage in close proximity, indicating a well-defined range of spread of infection by spores. During early summer the spots appear on fully expanded leaves about midway up the

plant, the young foliage higher up generally remaining free from attack. There is no doubt that many of the spots remain small, relatively inconspicuous, and innocuous, but under favourable conditions for the fungus they rapidly enlarge. They then assume their first distinguishing characteristic, namely, a basin-like depression becoming grey or whitish in the centre and surrounded by a slightly raised, chocolate-coloured ridge, defining the outer circumference of the attacked area. The ridge, roughly circular, is more definite when the spots are from 2–3 mm. in diameter. Larger spots have an entirely different appearance, having a less definite margin and commonly being surrounded by irregular extensions of blackened tissue indicating the rapid growth of the parasite in the leaf tissue (Pl. XIII, fig. 1). During wet weather infection may become active and the blackened areas may extend rapidly. At this stage the centre of the spot commonly appears silver-grey, and close examination will reveal a densely aggregated mass of projecting conidia. These may be found on both surfaces of the leaf but are more often formed on the upper surface. The production of conidia, however, cannot always be observed. Many spots do not appear to produce conidia, even under favourable conditions in the laboratory. Conidia are occasionally formed around the outer circumference of the spot and are characteristic in shape. They usually taper slightly at each end, often more so at the distal end, and they are sometimes slightly constricted at the septa. The conidiophore is relatively short.

Spotting or discoloration of leaves or stems of *Vicia Faba* may be caused in many ways, and confusion as to the identity of the cause may easily arise, for reaction to mechanical or other injury is rapid in the bean plant. Frost injury is easily identifiable because of the brown "scorched" patches which commonly occur on the most exposed leaves and stems—this being commonly accompanied by characteristic distortion. The brown streaks on the stems due to frost, however, may easily be confused with similar markings said to be characteristic of Chocolate Spot disease. In the early stages, foliage spotting by the latter (Pl. XIII, fig. 2) cannot easily be distinguished in the field from that caused by *Cercospora Fabae* or by *Ascochyta Fabae*; small brown spots equal in size and similar in appearance are produced by both (Pl. XIII, fig. 3), and the only means of discriminating between them is by careful laboratory examination. So far as Chocolate Spot disease is concerned, I am not aware of any dependable means of diagnosis, as the organism believed to cause it has not been isolated from the leaf spots. In this connection it is well to point out that Riker and Riker (6) have isolated from chocolate-coloured spots on broad bean leaves an organism resembling *Pseudomonas seminum* Cayley and vigorously pathogenic to broad beans and garden peas grown in the greenhouse. These writers point out, however, that

further study is necessary before a causal connection between this organism and the disease can be regarded as established. Minute spots may be produced by *Botrytis cinerea* on the blossoms (Pl. XIII, fig. 4), leaves, stems, and pods of field beans and it is necessary to take this into account, as at certain stages the symptoms produced are similar to those indicated above.

In the absence of spores, spots caused by *Cercospora Fabae* may easily be confused with those caused by *Ascochyta Fabae*, or by aphis injury. *A. Fabae* is distinguishable by means of the small circular whitish area of dead tissue, often glossy in appearance, which occurs in the centre of the older spots and on which the pycnidia of the fungus may usually be discerned (Pl. XIII, fig. 5). The injury caused by aphides (Pl. XIII, fig. 6) is remarkably similar to that caused by *C. Fabae* and *A. Fabae*, and it particularly resembles the spots with glossy whitish centres produced by *A. Fabae*. The photograph (Pl. XIII, fig. 6) of injury by aphides was obtained by allowing aphides to feed on a bean plant under a muslin cage. The injury shown in the photograph was done during a fortnight's feeding period. Concentric circles defining spots do not always accompany *C. Fabae*, and, in fact, they rarely occur except in large spots and in those resulting from artificial inoculation (Pl. XIII, fig. 7). In the field, leaves affected with *C. Fabae* often present a shot-hole effect, whereas this has not been seen in laboratory inoculations with the fungus.

The symptoms of infection by *C. Fabae* under greenhouse conditions are distinct from the symptoms seen in the field, the leaf spots in the former being devoid of brown or chocolate-coloured markings at all stages, and being characterised invariably by concentric circles around the periphery (Pl. XIII, fig. 7). Furthermore, the concave shape and the ridge have not been observed; the spot is dark grey, and spore production is more prolific.

C. Fabae is not entirely confined to leaves, although the most obvious symptoms of the disease are produced on them. Black lesions at the junction of branchlets with the main stem are not uncommon, and also on the main stem itself during spring on young plants about to begin, or during the early stages of, active growth. Conidia of *C. Fabae* have been found on affected stipules accompanying such lesions.

The examination of leaf tissue in the region of spots is a ready means of distinguishing *C. Fabae* from *A. Fabae*. The chief distinguishing characteristic is the presence of pycnidia embedded in the tissue in the spots caused by *A. Fabae*. In the absence of mycelium, aphid injury or Chocolate Spot should be suspected.

III. The fungus

Cercospora Fabae was first described by Fautrey (2) from material collected in 1890 at Clamercy, Côte-d'Or. Keissler (3) drew attention to the characters distinguishing it from the closely related *C. zonata* Winter, which occurs on the same host. Keissler regarded *C. Fabae* Fautr. as distinct from *C. zonata* Wint. from differences in the descriptions of the two species, but was unable to come to a satisfactory conclusion from the examination of specimens, as the spots on Fautrey's specimens were sterile. However, there seems no real reason for separating the two species. It is true that Fautrey describes *C. Fabae* with conidia having 7–9 septa and violet-coloured conidiophores, whereas Winter (9) describes *C. zonata* as having 4-septate conidia and dark hyphae. Also the sizes of the conidia and conidiophores differ, being 60–110 × 5–7μ and 60 × 6μ respectively in *C. Fabae* and 40–65 × 4–6μ and 26–44 × 6–8μ respectively in *C. zonata*. Examination of *C. Fabae* in Roumeguère's set of Fungi Selecti, which appears to be Fautrey's original collection, and comparison with *C. zonata* Wint. collected at Coimbra in 1883, specimens of both of which are in the herbarium of the Royal Botanic Gardens, Kew, yielded little information as both are sterile. The colour and shape of the spots are, however, identical. Examination in 1932 of freshly gathered material of the ordinary field bean grown in England showed the average dimensions of fifty conidia to be 75 × 4·7μ with extremes of 40–126 × 4–6μ. The number of septa varied from 3–7, the average number being 7. The common number in mature conidia was 7. Examination in October 1929 of conidia produced in a cool greenhouse after inoculating leaves of *Vicia Faba* from a monosporous culture showed the average length of 100 conidia to be greater, namely, 93 × 4·5μ with extremes of 44–171 × 3·5–5·5. The number of septa varied between 1–14, the average number being 7. The difficulty of obtaining comparable characteristics of the species is thus apparent; this was pointed out by Welles (8) to apply to other members of the genus. He found that conidia may be 50–150 per cent. longer when produced during the rainy season, and stressed the importance of the critical comparison of species on artificial media and of the degree of their parasitism on the host. Until living material is compared and critically examined on artificial media and on the host it appears reasonable to believe that *C. Fabae* Fautr. and *C. zonata* Wint. may be identical.

C. Viciae Ell. and Hol. (1), a species occurring on *Vicia sativa* in America, is probably distinct, as the dimensions of conidia and number of septa do not approach those of the species under consideration.

IV. Inoculation trials

A monosporous culture of *Cercospora Fabae* was obtained without difficulty from material collected in the field in January 1927. It was found necessary, however, to place the infected plants under a bell-jar for a few days in order to allow the production of spores, before attempting to isolate the fungus. The fungus grew well on potato agar. On February 18th five inoculations were made with an accompanying control on the same leaf, by means of needle pricks; inoculum from the pure monosporous culture on potato agar was used for the inoculations and a sterile needle for controls. On March 4th leaf spots typical of *C. Fabae* had been produced at the five points of inoculation, whereas controls remained uninfected. The inference drawn from this trial was that *C. Fabae* readily infects leaves of field beans through needle prick inoculations.

On June 28th, 1929, another monosporous culture of *C. Fabae* was obtained from material collected in the field from field beans. A monosporous culture of *Cercospora cantuariensis* from a culture kindly supplied by Dr Wormald, East Malling, was also obtained on July 2nd, and the two species of *Cercospora* were compared in a series of inoculation trials on broad beans. Attempts at inoculation with each fungus were made on leaves by means of a needle scratch and on uninjured leaves. All the plants were kept under bell-jars for a few days following inoculation. On August 2nd eighteen inoculations were made with *C. Fabae* on wounded leaves and the same number on unwounded leaves with the same number of controls. A week later leaf spots typical of *C. Fabae* appeared (Pl. XIII, fig. 7) at all (eighteen) needle scratch inoculations, whereas no infections occurred on the eighteen uninjured but otherwise similarly inoculated leaves. On August 2nd, also, a similar series of trials was carried out using *C. cantuariensis*. Of nine inoculations on injured leaves two were successful on August 9th, the nine controls being uninfected. No infections arose from the inoculation of nine uninjured leaves. On September 4th these trials were repeated on both field beans and broad beans. Examination on October 7th revealed that with *C. Fabae* six small infections on uninjured leaves of field beans had occurred out of a total of forty-two inoculations, whereas on injured leaves forty-one infections out of forty-two inoculations were well established. On broad beans one small infection arose from the inoculation of twenty injured leaves. *C. cantuariensis* produced on injured leaves of field beans twenty-five slight infections out of twenty-eight inoculations. Little growth of fungus was apparent, but the infections which did arise were distinct. No infections resulted from the inoculation of nine uninjured leaves.

The inference drawn from these trials was that *C. Fabae* readily

infects injured leaves of field beans, confirming the 1927 trials; that infection resulting from the inoculation of uninjured leaves does not occur frequently on field or broad beans under artificial experimental conditions, and that such infection as does occur is limited and inactive. *C. cantuariensis*, on the other hand, does not readily infect injured leaves of field beans, although what approximates to "sub-infection" occurs, namely, the tolerance, at least for a few weeks, of small, limited spots. On uninjured leaves of field beans *C. cantuariensis* does not become established.

A supplementary trial in which conidia of *C. Fabae* were used was also carried out. The conidia were obtained from active lesions on leaves resulting from monosporous inoculations, and they were applied to both injured and uninjured leaves in drops of sterile water. Seven injured leaves of field beans and the same number of uninjured leaves were inoculated on October 12th, 1929. The injured leaves were inoculated on both surfaces. On October 15th spots typical of *C. Fabae* appeared at the points of inoculation on four out of seven leaves. No infection occurred on uninjured leaves or on uninoculated controls.

The inference drawn from this trial is that spores of *C. Fabae* readily infect leaves of field beans by way of wounds and that, at least under artificial conditions, uninjured leaves do not become infected.

Further inoculation trials were undertaken to discover whether *C. Fabae* attacks the stem of the bean plant. Black soft lesions are commonly found on stems of young infected plants during early spring and often result in their death, but spores of *C. Fabae* have not been observed on the stem itself, although they have been found on infected stipules associated with lesions. On March 28th, 1930, stems about one to two inches above soil level were inoculated under the usual aseptic conditions. Damp cotton-wool and tin-foil were bound round the stem at the point of inoculation. On April 28th small lesions were observed at the point of inoculation on two out of three plants which had been injured. The lesion was confined to the epidermis and had extended to the stipules. The two uninjured and inoculated stems remained uninfected as well as the two injured controls. On May 8th the lesions were one inch in length. On April 24th the experiment was repeated except that no wool or tin-foil was used as it was thought that this might be conducive to the blackening of the stem. Inoculation was done with a needle, one inch above soil level, using an agar culture of *C. Fabae*. The plants were kept for four days under a bell-jar. There was no sign of infection on May 2nd, but on May 8th a typical lesion, slightly sunken and parasitic, appeared.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 7

Fig. 6

V. Summary

1. *Cercospora Fabae* Fautrey was observed on field beans on January 15th, 1927, at South Leigh, Oxfordshire. This is believed to be the first report of its occurrence in England.

2. The fungus is widely distributed in England.

3. It causes spotting of the foliage, which becomes more severe during damp and warm weather, but it may be found on field beans throughout the year.

4. The symptoms produced are chocolate-coloured spots on the leaves, which are described and compared with closely resembling symptoms produced by *Bacillus Lathyri*, *Ascochyta Fabae*, injury by aphides, frost injury, and *Botrytis cinerea*.

5. Conidia cannot be observed easily on all spots resulting from *C. Fabae*, but when present may be in a sorus which is silver grey in colour and is situated on dead tissue; alternatively, conidia may occur around the outer circumference of the spot.

6. Mycelium and spores from a monosporous culture of *C. Fabae* readily infect injured leaves of field beans and, under conditions of experiment, do not so frequently infect uninjured leaves. *C. cantuariensis* grown for comparison appeared to be a very weak wound-parasite on leaves of field beans.

7. *C. Fabae* is believed to produce black lesions on the stem of field beans, and this was confirmed in part by inoculation.

It is a pleasure to acknowledge assistance with references kindly given by Miss E. M. Wakefield, Dr G. H. Pethybridge and Mr W. C. Moore.

Explanation of Plate XIII

Fig. 1. Typical spots produced by *Cercospora Fabae* on leaves of *Vicia Faba*. Note the less defined spots and the extension of the fungus in the leaves on the right hand at the bottom. × ¾.

Fig. 2. Chocolate Spot of beans. Chocolate-coloured spots on bean leaves alleged to be due to *Bacillus Lathyri*. Natural size.

Fig. 3. *C. Fabae*. Small chocolate-coloured spots. A later stage is shown in Fig. 1. × ½.

Fig. 4. Dark-coloured spots produced by *Botrytis cinerea* on blossoms of the bean plant. Natural size.

Fig. 5. *Ascochyta Fabae*. Typical spots produced by this fungus on bean leaves. Note the pycnidia in the central white portion of the spots. × 1¼.

Fig. 6. Chocolate-coloured spots produced by allowing aphides to feed on bean leaves.

Fig. 7. *C. Fabae*. Typical, dark grey spots with concentric circles resulting from the wounding and inoculation of top pair of leaves. The pair of leaves at the bottom acted as controls.

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COMMON NAMES FOR PLANT DISEASES

(The following article has been prepared by a sub-committee of the B.M.S. Committee for Plant Pathology and has been approved by this Committee for publication)

The Society's Committee for Plant Pathology has read with considerable interest the article entitled "Standardization of Common Names of Plant Diseases" by Dr G. H. Cunningham of New Zealand, published in vol. xvi of the *Transactions* (1931), pp. 145–148. Since some important points are raised, it is considered that a reply to some of Dr Cunningham's criticisms is required; and the following observations are made to this end.

In the Committee's view the primary object of fixing a standard common name for each plant disease is to provide *growers* and other "laymen" with a designation or label for each disease, which they can use both amongst themselves and in their contacts with plant pathologists. That plant pathologists also will use such names amongst themselves is likely, and not undesirable, especially when the names become fully standardised; but it is for growers rather than for plant pathologists that standard common names are particularly required.

Before going further it is necessary to emphasise the point that it is the *diseases* that are to receive common names, and not the pathogens concerned (if any). Unfortunately many plant pathologists persist in using the name of a pathogen as if it were the name of a disease it causes. Attention has already been called in these *Transactions* (xii (1927), 95) to a list of so-called "Foreign Plant Diseases" new to, or not widely distributed in, the United States of America, which is nothing of the kind. More recently "A Check List of Plant Diseases and Fungi occurring in Egypt" has been published*, covering more than sixty pages of print; but if there is one single name of a plant disease in this list it is hard to find, except on the last three or four pages where "Plant diseases other than fungous or bacterial" are dealt with.

Now, in recognising a disease in his plants it is generally the abnormal aspects they display (*i.e.* the disease *symptoms*) that attract the attention of the grower, and therefore common names should, as a rule, be based on symptoms. Sometimes, however, the parasite itself or some part of it is the most conspicuous abnormality present, and in such instances there is no reason why the common name of the disease should not have reference to the features presented by the parasite, or should not include the common name (if one exists) of the group of pathogens (*e.g.* Rust) to which the one concerned

* L. E. Melchers, *Trans. Kansas Acad. of Science*, xxxiv (1931), 41–106.

belongs. To introduce the scientific name of the parasite into the common name is, however, at least undesirable, although it must be confessed that this has been done in the Committee's list in a few instances.

Dr Cunningham objects to the derivation of the common names from host symptoms but does not appear to divorce disease from pathogen in his mind. He thinks that the choice of symptoms resulting from the attacks of the various pathogens is too limited, but he forgets that when speaking of a plant disease the name of the host plant is invariably mentioned. What is required is not a separate common name for each of the diseases to which the whole vegetable kingdom is heir, but as far as possible a different and specific common name for each disease that affects a given host. Not only is there no reason for rejecting such names as Mildew, Blight, Canker, etc., but these names, and others like them, are, for more than one reason, often the most suitable ones to standardise in relation to specific hosts. There can be no objection, for example, to speaking of Apple Canker and of Larch Canker, although the pathogens concerned are not identical. Similarly, although the pathogen is different in each case, the common names Potato Scab, Apple Scab, Gladiolus Scab and Peach Scab are perfectly legitimate and satisfactory for the respective diseases, provided the use of them is standardised. In the Committee's list the common names of the diseases suggested for standard use are purposely assembled in each case under the host, and it is to be assumed that when speaking of a disease the name of the host will be mentioned.

Dr Cunningham suggests that common names should be instituted for individual pathogens. The necessity for this is not clear; at any rate the Committee's object was to settle common names for *diseases*, not for pathogens. On the other hand, the common names of groups of pathogens, such as Rust, Smut, Mildew, etc. are often incorporated in the common names of diseases.

It is true, as Dr Cunningham remarks, that there are no principles governing the use of common names. Such names have simply accumulated in course of time in more or less haphazard fashion. Nevertheless, in drawing up the list, the Committee did follow certain rules, although these were not adhered to with absolute rigidity. Compromise is sometimes the only path to progress. Thus, it was agreed that as few names as possible should be created; that one or other of the existing names should be employed, except in a few cases where they were quite inappropriate or even misleading and that where more than one common name for a specific disease already existed, the one which the Committee believed to be longest established or most widely used amongst growers (not necessarily amongst plant pathologists) should be selected.

Dr Cunningham objects to the use of different common names for different diseases, as exhibited by different symptoms in the host plant, or its parts, when one and the same pathogen is concerned. This principle, however, was deliberately adopted by the Committee, and if Dr Cunningham would envisage a disease as an entity and lose sight for a moment of the pathogen, he would see the reasonableness of this principle. Growers recognise diseases by their symptoms; they know little or nothing about the pathogens.

Bearing in mind what has already been said as to the association of the name of the host with the common names of the diseases that attack it, it is not correct to say that in the Committee's list one disease has been attributed to several pathogens in the cases cited by Dr Cunningham. Leaf Spot of Strawberry, for instance, is not the same disease as Leaf Spot of Celery; the Mildews (this name was deliberately adopted where the *Erysiphaceae* are concerned, and Downy Mildews for the Phycomycete types) of twenty or more different hosts are not identical diseases; the various Downy Mildews, Rusts, Smuts, etc. are not one and the same disease on a large number of hosts. On the other hand Damping Off is an example of a disease that may be caused by more than one pathogen, and may, moreover, occur in several hosts. Nevertheless, the Committee had no hesitation in retaining this common name.

The example of the polyphagous pathogen *Sclerotinia Sclerotiorum* which Dr Cunningham selects for comment does not, as a matter of fact, produce similar symptoms on all its hosts as he suggests. Thus, it causes a rot of artichoke tubers (the most familiar complaint with this host in this country); it does not, under natural conditions, rot potato tubers but causes collapse of the stalks; in Antirrhinums it causes a wilt of the flowers, and so on; all different diseases, showing different symptoms and therefore requiring different common names. Nor has the pathogen acquired, as Dr Cunningham states, several names in the list; it is referred to in all cases there as *Sclerotinia Sclerotiorum* (Lib.) de Bary!

Phoma Lingam in England causes two distinct diseases in Swedes. In the seed-producing crop it causes what is always known by growers as Canker; in the root crop it produces Dry Rot of the "root" or "bulb." There appears to be no reason why these two well-established common names should not be retained, although the pathogen concerned is the same in the two diseases. Attacks by this fungus on cabbages in England are not common, and it may perhaps be admitted that it would have been desirable to apply the name Canker to such attacks rather than Stem Rot.

But the difficulty in the adoption of Dr Cunningham's views becomes apparent when he seems to suggest that the Die Back of Rhododendrons, caused by *Stereum purpureum*, ought to be called

Silver Leaf. True, most of the hosts attacked by this parasite show Silver Leaf symptoms, which result from a very definite pathological condition of the tissues of the leaf. But for a plant pathologist to tell a grower that his Rhododendrons are suffering from Silver Leaf, just because they are attacked by *S. purpureum*, and when there is no sign whatever of a silvered condition in the foliage, would be to invite derision.

The Committee agrees with Dr Cunningham that appropriateness in a common name for a plant disease must receive due consideration, and it was because *Cystopus* is not a Rust and does not look rusty that it was decided, not without some regret, that the old name of White Rust for the disease of crucifers caused by *C. candidus* was inappropriate, and White Blister was substituted for it. It was the Committee's decision that the name Rust should be incorporated in a common name of a disease only when the pathogen concerned was a member of the Uredinales.

Spraing (more commonly spelled Sprain in the past) is an appropriate name for the disease of the potato tuber that it connotes, both from the point of view of meaning and derivation; moreover, it is one that has long been in use amongst potato growers. Until the causes of the various internal brown necroses of potato tuber tissue have been more fully and explicitly determined it seems ill advised to discard this useful common name.

Blight, when applied to potatoes in this country has indeed long had specific application to the disease caused by *Phytophthora infestans*. In the British Isles, Late Blight is an entire misnomer for this disease, for it occurs early (April) in some parts of England*, and there is no potato disease here showing a similar type of symptoms that occurs earlier. Even in America, where the name Late Blight was first suggested for the disease, it is not altogether appropriate. It often occurs there as early as or earlier than the so-called Early Blight (*Alternaria*) and the originator of the name Late Blight has admitted that he much doubts the wisom of having suggested it.

Nothing perhaps can be said in defence of the name Crown Rust, for it is connected with a morphological feature of the pathogen which can be seen only with the microscope. Farmers, however, seldom discriminate in this country between the different types of Rusts on their cereals, and it is to be feared that the name in question was adopted by the Committee with the mycologist in view rather than the farmer. It is to be admitted that in general the microscopical features of pathogens are inappropriate for incorporation into the common names of diseases.

* In 1932, potato Blight was seen as early as January on the foliage of "ground-keepers," or "volunteer" plants, growing amongst the bulb (Narcissus) crop in W. Cornwall.

All will not agree with Dr Cunningham that the name Finger and Toe is not so descriptive of the disease in crucifers caused by *Plasmodiophora Brassicae* as is the name Club Root. Usage, in this country, is about equally divided between the two names. The Ministry of Agriculture's Report (Miscellaneous Publications, No. 52), which was used as the basis of the Committee's list, employs the former name, and so does the Leaflet on the disease issued by them. Hence this name was retained.

In condemning the use of the "-oses" Dr Cunningham is, so far as the Committee is concerned, flogging a dead horse. True, Anthracnose occurs in the Committee's list as a common name in several instances; but its sponsors had to make a hard fight for its retention.

The Committee, in preparing the list, was not unaware of the difficulties of the task and, as stated in the introduction, regarded the list as to some extent provisional and tentative. It did not venture to hope that the list itself would be acceptable to all parts of the Empire in which the diseases listed occur, but the hope was expressed that similar lists would be drawn up in other parts of the Empire. When this has been done—and Canada has already made a start, for the Canadian Phytopathological Society has appointed a Committee to prepare a list of the common names of plant diseases suitable for use in Canada*—the various lists can be compared and correlated. Ultimately, presumably under the aegis of an Imperial Mycological Conference, it may perhaps be found possible to consolidate the lists into a single one, which may become the standard for Empire needs. Towards this goal the Committee for Plant Pathology of the British Mycological Society will be glad to work in co-operation with similar bodies overseas as Dr Cunningham suggests.

* *Proceedings of the Canadian Phytopathological Society*, Inaugural Session, 1929, Ottawa, 1930, p. 15.

SPECIES OF *MORTIERELLA* ISOLATED FROM SOIL

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(With 8 Text-figures)

Among the species of soil fungi isolated from a sandy loam in Victoria (4, 5), eleven belong to the genus *Mortierella* Cöemans. Of these five could not be identified as species which had been previously described.

Historical

In their memoirs on the Mucorinae, van Tieghem and Le Monnier (18, 19, 20) gave detailed descriptions of ten new species of *Mortierella* bringing the total number in the genus up to thirteen. One species, *M. simplex* van Tieg., was found growing on damp soil.

The species, *M. repens* A. L. Sm. and *M. Baineri* var. *Jenkini* A. L. Sm., were recorded by A. L. Smith (14) as growing on soil.

It was not until later that species of *Mortierella* were isolated as constituents of a soil flora. Oudemans and Koning (8) obtained four species from soil in Holland; *M. pusilla* Oud., *M. humicola* Oud., *M. subtilissima* Oud. and *M. isabellina* Oud. J. Dauphin (3) gave descriptions, taken from the original papers, of the twenty-nine species of *Mortierella* then known. He so arranged the forms that the relationships of the species could be seen according to the mode of branching of the sporangiophore. Many species he grouped as "petites espèces" and "variétés" of the well-defined species which he terms "grandes espèces."

Saccardo records three new species since Dauphin's monograph, two described by Peyronel (9) and one by Rostrup (12). *M. pusilla* Oud. was recorded from soil by Janke and Holzer (7), and a variety of this species was isolated from the soil of a salt marsh by Bayliss Elliott (1).

Four new species have been recently described. One, *M. nivea velutina*, was isolated from the skin by Ciferri and Ashford in Porto Rico (2). Another, *M. elasson* Sideris and Paxton, was found on decayed pineapple roots (13). *M. atrogrisea* was named by van Beyma from decaying birch wood (17). *M. humillissima* Pispek is the only new species which has been recorded from the soil (10). Unfortunately the paper by Pispek was not obtainable in Australia.

The above are the only species of *Mortierella* which I have been able to trace in the recent literature.

Generic description of *Mortierella*

Mycelium very thin and delicate, white or light coloured; nutritive mycelium branched and irregularly swollen, containing oil droplets; aerial mycelium branched and fine.

Sporangiophores erect, simple or branched, often tapering towards the apex. Sporangia terminal, usually spherical, without a columella; membrane diffluent often leaving a small basal collar. Spores numerous, small, spherical or oval, sometimes irregular.

Chlamydospores developed in nutritive mycelium, either intercalary or terminal.

Stylospores or aerial chlamydospores formed on short aerial branches, spherical, thick walled often with characteristic markings.

Zygospores enclosed in a thick wall formed of intertwined sterile hyphae.

The reproductive organs characteristic of the genus do not occur in all species and their presence may be dependent on the type of culture medium. The amount of colour developed in the mycelium or in the sporangium when the spores are ripe also varies according to the food materials present.

Culture media

The fungi were grown from a very dilute soil suspension, on plates of Brown's synthetic agar and transferred to test-tube slopes of Czapek's agar. This medium did not usually give luxuriant growth and other media were used for a more detailed examination.

Dauphin's glucose-peptone medium (3) made up with agar or with gelatine gave increased growth in mycelium and reproductive organs.

Beerwort gelatine was used by Oudemans and was satisfactory, except that the gelatine was liquefied as the colony aged.

Cornmeal agar (Sideris and Paxton (13)) induced the formation of mature zygospores.

Description and systematic account of five new species of *Mortierella*

(The descriptions are based on cultures on Czapek's medium)

1. *Mortierella isabellina* Oud. var. *ramifica* nov.var. (Text-figs. 1–4.)

Growth slight, colonies small, scattered. At first white, becoming light drab grey (Ridgway, Pl. XLVI). Hyphae more or less regular and sometimes forming chlamydospores. Sporangiophores occasionally simple, usually branched, forming broom-like clusters 300μ in height. Protoplasm homogeneous; branches 1·5μ diam., straight, tapering to the apex. Sporangia brown, 18μ diam.; collar left on

sporangiophore; spores irregularly globose, smooth, 2·5–3μ in diam. No stylospores or zygospores observed.

Mycelio exiguo, acervatim sparso, primum albido, demum subcinerascente; hyphis ± regularibus, chlamydosporas interdum praebentibus; hyphis sporangiferis interdum simplicibus, sed plerumque scopaeformiter ramosis, 300μ altis, protoplasmate homogeneo; ramis 1·5 diam., rectis, versus apicem sensim attenuatis; sporangiis fuscis, 18μ diam., collari basilari persistente; sporis irregulariter globosis, levibus, 2·5–3μ diam. Stylosporis et zygosporis haud observatis.

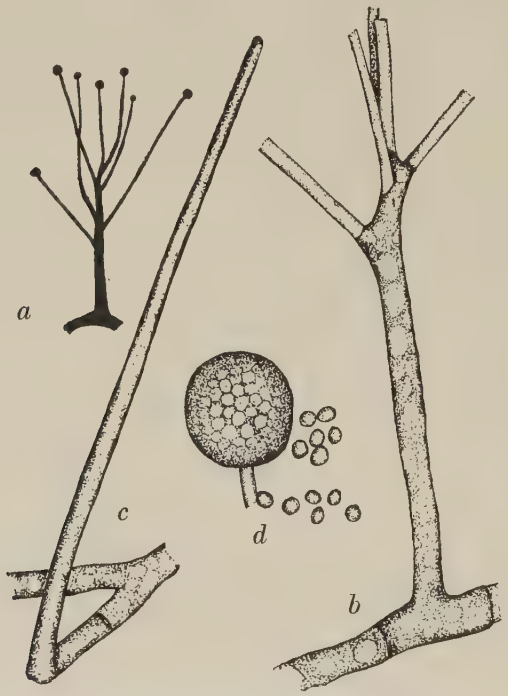

Fig. 1. *Mortierella isabellina* Oud. var. *ramifica* nov.var. *a*, mode of branching of sporangiophore; *b*, *c*, details of the sporangiophore (Czapek's medium); *d*, sporangium and spores (Czapek's medium). (× 625.)*

This form was isolated from bush soil in 1929.

The mycelium appears white at first on all media. On glucose-peptone agar and wort gelatine there is a dense white felt. The white changes to a light drab grey, as the sporangia are produced. The colony on wort gelatine shows wrinklings and zoning on the reverse side.

* All drawings except diagrams of sporangiophore branching were made under high power by camera lucida.

The hyphae form chlamydospores on wort gelatine and become swollen and irregular on glucose peptone. They are more or less regular and contain oil droplets when grown on Czapek's medium. The branches are less elongated on this than on other media. On some media a slight swelling occurs below the sporangium.

Cultures from another isolation differed slightly from those just described. They were much paler in colour, especially on wort gelatine. Chlamydospores developed on Czapek and the hyphae were very

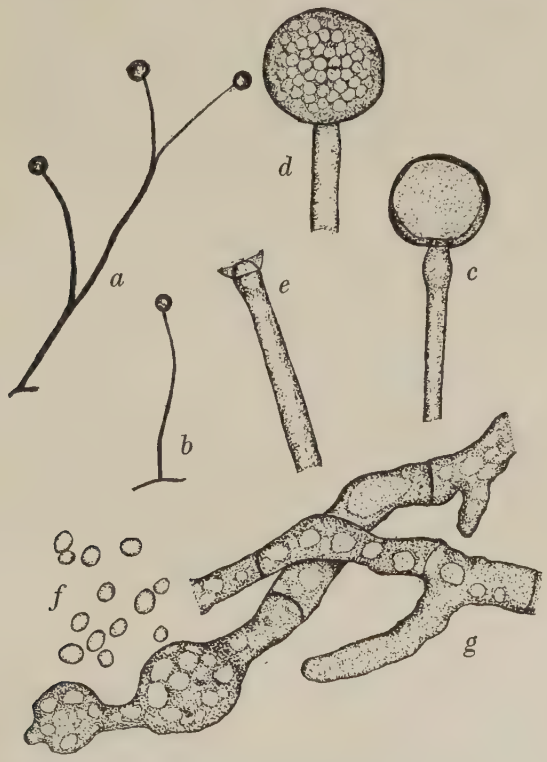

Fig. 2. *Mortierella isabellina* Oud. var. *ramifica* nov.var. *a*, branched sporangiophore; *b*, single sporangiophore; *c*, *d*, showing swollen sporangiophore; *e*, collar on sporangiophore; *f*, spores; *g*, hyphae; *a–f*, glucose-peptone agar; *g*, wort gelatine. (× 625.)

irregular on glucose-peptone agar. The sporangiophores were shorter and sporangia smaller, while the spores were more regular in size, 2·5μ in diam. (Fig. 3).

Cultures from a third isolation showed the same cultural characteristics, but on Czapek's medium chlamydospores were very numerous. The sporangia were about 20μ in diam., the spores were regular in size (Fig. 4).

The fungi from these three isolations, although differing in minor respects, can be grouped as strains of a single species, resembling *M. isabellina* Oud. in the grey colour developed in cultures on wort gelatine, and in the dimensions of the sporangia and spores. The absence of branching in the sporangiophore of *M. isabellina* is a definite character which Oudemans described for all his species from the soil. Although isolated simple sporangiophores occur in this form, the branching is very characteristic.

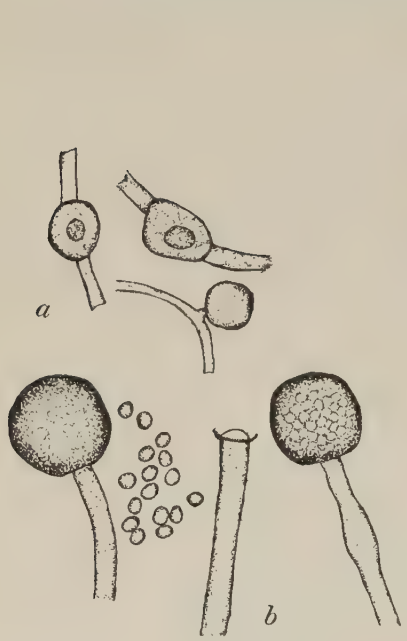

Fig. 3.

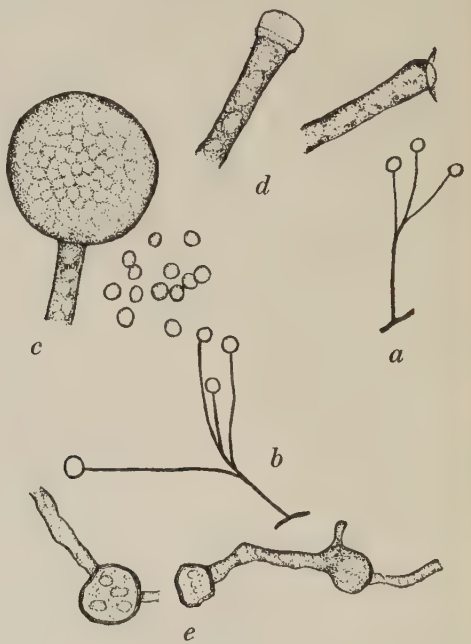

Fig. 4.

Fig. 3. *Mortierella isabellina* Oud. var. *ramifica* nov.var. *a*, chlamydospores (Czapek's medium); *b*, sporangia and spores (wort gelatine). (× 625.)

Fig. 4. *Mortierella isabellina* Oud. var. *ramifica* nov.var. *a*, *b*, mode of branching; *c*, *d*, sporangiophores and spores; *e*, chlamydospores. *a–d*, wort gelatine; *e*, Czapek's medium. (× 625.)

M. atrogrisea van Beyma (17) is evidently much darker in colour but has the same type of branching and similar measurements for sporangia and spores; van Beyma stresses the absence of chlamydospores. He described the formation of successive sporangia, a phenomenon not observed in this species, which I regard as a variety of *M. isabellina*.

2. *Mortierella vinacea* nov.sp. (Text-fig. 5.)

Mycelium forming a thick cottony felt, becoming russet vinaceous, (Ridgway, Pl. XXXIX), as the sporangia mature. Chlamydospores formed, the hyphae irregularly swollen. Sporangiophores racemosely

branched, 4μ diam. Sporangia brown, 20μ diam.; no collar formed. Spores 3–5μ, irregularly angular, slightly coloured. No stylospores or zygospores.

Mycelia dense contexto, russo-vinaceo quum sporangia maturescunt, chlamydosporas et tumidas hyphas praebente quum in "Czapek's Medium" colitur; hyphis sporangiferis racemose ramosis, 4μ diam.; sporangiis fuscis, 20μ diam., collari basilari haud persistente; sporis 3–5μ diam., irregulariter angulatis, parum coloratis. Stylosporis et zygosporis ignotis.

This species was isolated from cultivated soil during 1928, and from bush soil in 1929.

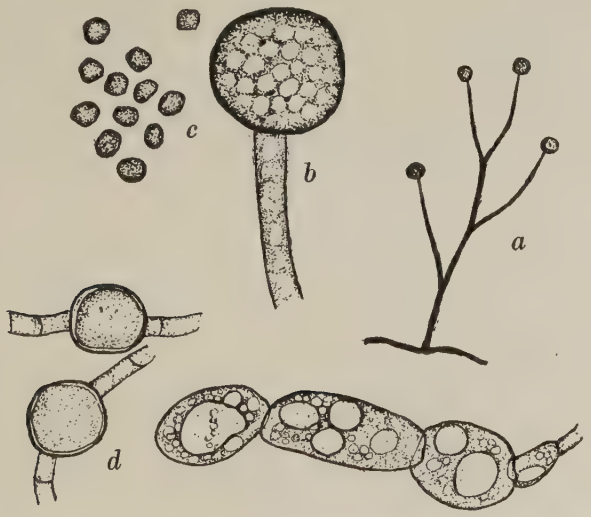

Fig. 5. *Mortierella vinacea* nov.sp. *a*, branching of the sporangiophore; *b*, *c*, sporangium and spores; *d*, chlamydospores and swollen hyphae. Czapek's medium. (× 625.)

A thick cottony felt is formed on all media. The russet-vinaceous colour is deepest on glucose-peptone agar. Wort gelatine is gradually liquefied and becomes discoloured, the colony showing a great deal of wrinkling.

On Czapek's medium, chlamydospores are formed on nutritive hyphae and numerous swellings occur on some hyphae (Fig. 5 *d*).

The second isolation of this form has more colour in culture.

This fungus differs from the described species in colour and in the type of branching of the sporangiophore. It is probably nearest to *M. isabellina* Oud. in the sizes of the sporangia and spores.

It differs from *M. isabellina* var. *ramifica* in being much more vigorous, different in colour, larger, in having no collar, and in its spores being very irregular in shape.

The trivial name has been given on account of the coloured sporangia.

3. *Mortierella longicollis* nov.sp.

Mycelium a cottony felt becoming vinaceous as the sporangia mature, the colour being deeper when the mycelium is disturbed. Chlamydospores scanty. Sporangiophores elongated, slightly branched, 5–7μ in diam. Sporangia purplish brown, globose, usually with a neck-like base down which the spores extend to as much as 80μ, diam. 30μ. Spores brownish, small and angular, 1·5–2μ in diam. No stylospores or zygospores.

Mycelio contexto, fusco-vinaceo quum sporangia maturescunt, acutiusque colorato quum turbatum est; chlamydosporis paucis; hyphis sporangiferis elongatis, parum ramosis, 5–7μ diam.; sporangiis purpureofuscis supra globosis 30μ, et infra plerumque cylindrico-prolongatis; sporis subfuscis, parvis, angulatis, 1·5–2μ diam., et sporangium et collum occupantibus. Stylosporis et zygosporis ignotis.

This form was isolated from garden soil in 1928 and from bush soil twice in 1929.

The colour of the mycelium on wort gelatine is deeper than on other media, and is slight on glucose-peptone agar.

On Czapek's medium a few chlamydospores are formed in the nutritive hyphae.

Sometimes the wall of the sporangium is persistent at the base (Fig. 6 *e*).

The spores are brownish in mass, but have a greenish tinge when isolated.

The sporangia of this species are very distinctive and unlike those of any described species in colour and neck-like base.

The trivial name *longicollis* has been given on account of the elongated neck to the sporangium.

4. *Mortierella renispora* nov.sp. (Text-fig. 7.)

Mycelium always white; fine, sparse, cottony. Chlamydospores and swellings occur on the nutritive hyphae. Sporangiophores simple, coarser than the hyphae, with large basal foot formed from the original hypha. Height 200μ, tapering from 10μ at the base to 3μ at the apex. Sporangia colourless, walls evanescent, diam. 25μ; with basal collar. Spores 4 × 2μ, slightly kidney shaped, hyaline. Stylospores absent. Zygospores not well developed on Czapek's medium. Corn-meal agar gives well-formed spores with few investing hyphae. Zygospore bright brown, coat irregularly roughened, diam. about 30μ without hyphal coat.

Mycelio semper albido, tenui, exiguo, sub cultura chlamydosporas et hyphas tumidas praebente; hyphis sporangiferis simplicibus, quam

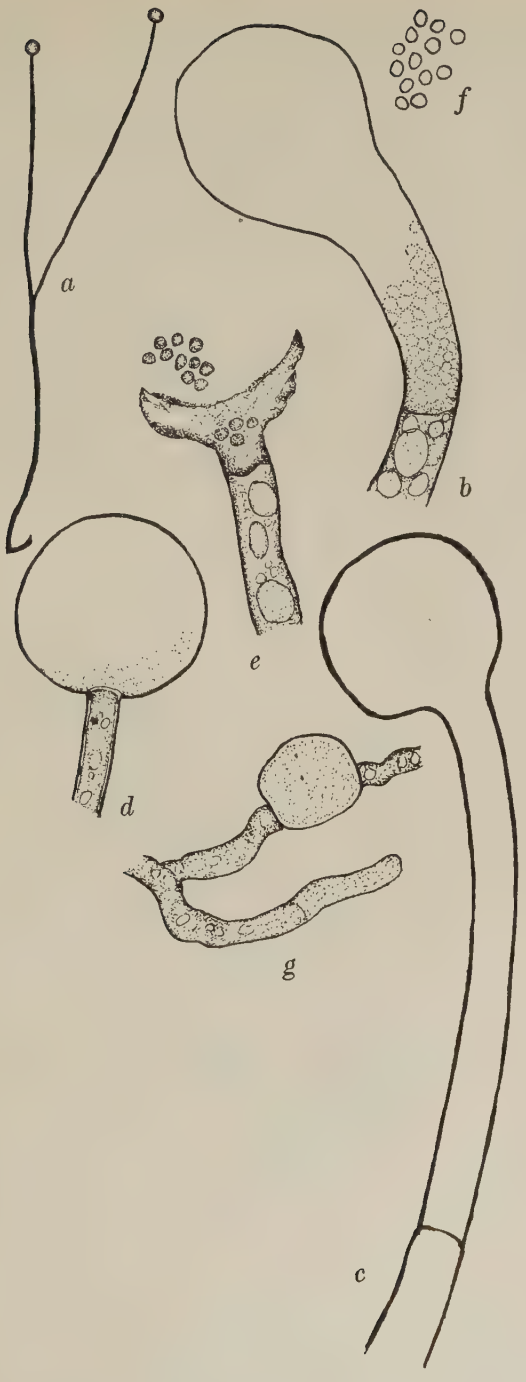

Fig. 6. *Mortierella longicollis* nov.sp. *a*, branching of the sporangiophore; *b*, *c*, sporangia with elongated necks; *d*, sporangium without the neck; *e*, persistent wall of the sporangium; *f*, spores; *g*, chlamydospores. Czapek's medium. (× 625.)

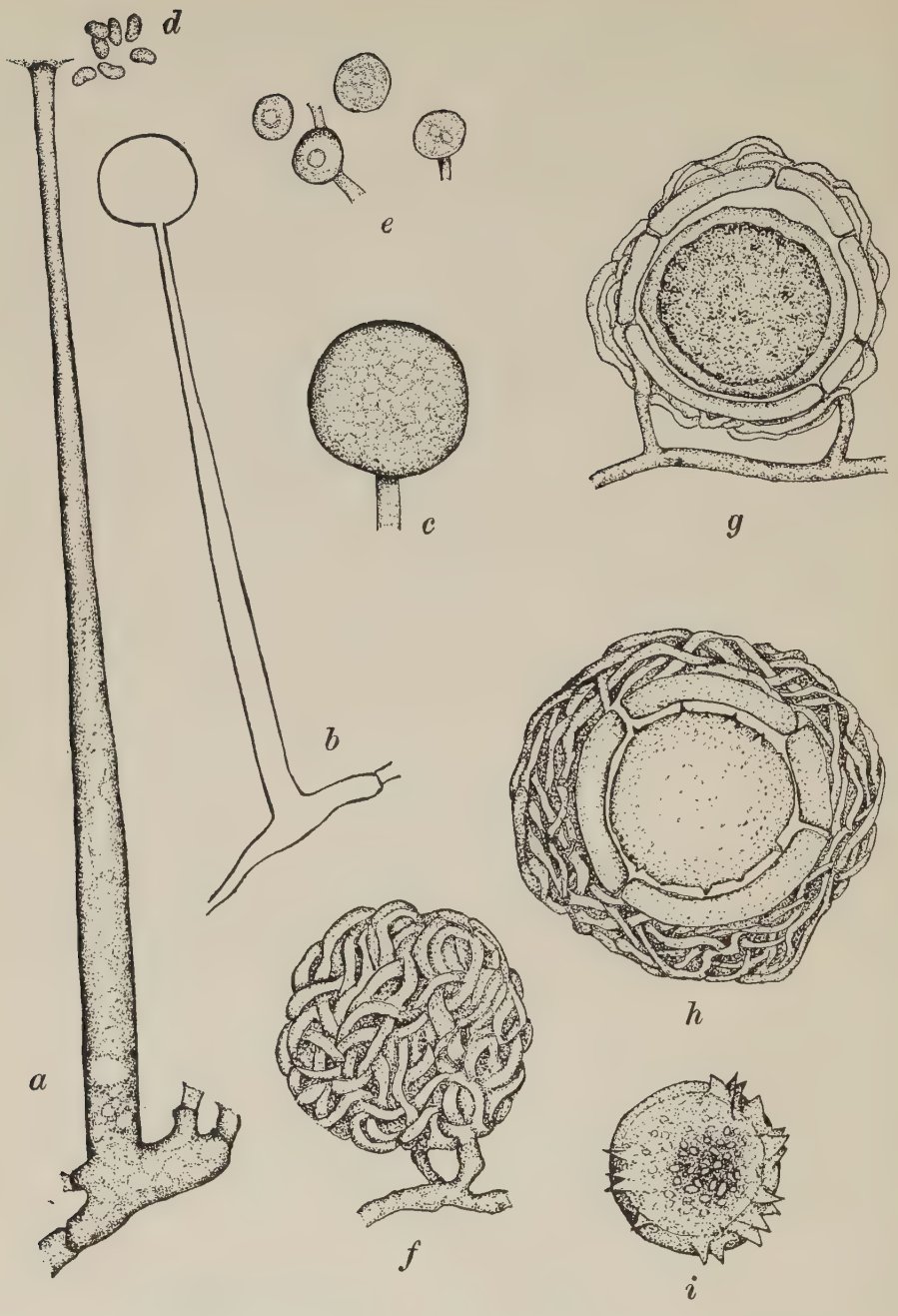

Fig. 7. *Mortierella renispora* nov.sp. *a*, *b*, sporangiophores; *c*, sporangium; *d*, spores; *e*, chlamydospores (× 625); *f*, zygospore (× 100); *g*, *h*, zygospores in optical section (× 500); *i*, zygospore without hyphal coat (× 500); *a–f*, Czapek's medium; *g–i*, corn-meal medium.

hyphis crassioribus, hypha basali inflata suffultis, 200μ altis, infra 10μ versus apicem 3μ diam.; sporangiis pallidis, 25μ diam., cuticula evanescente et collari basilari praeditis; sporis 4 × 2μ, subreniformibus, hyalinis. Stylosporis ignotis. Zygosporis in "Czapek's medium" sparse, in "corn-meal agar" bene evolutis, hyphis investientibus paucis; zygospora fulva, irregulariter aspera, 30μ diam.

This species was isolated from bush soil, once in 1928 and again in 1929.

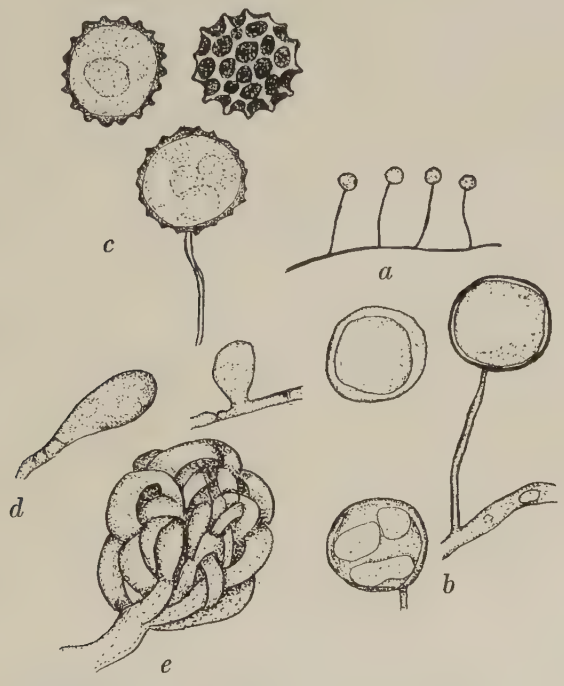

Fig. 8. *Mortierella stylospora* nov.sp. *a*, stylospores formed on aerial hyphae; *b*, *c*, stylospores; *d*, chlamydospores; *e*, zygospore. Czapek's medium. (× 625.)

The colonies are always pure white. There is a very fine cottony growth on Czapek agar, but on a glucose-peptone agar slope growth is so dense almost to fill the lumen of the tube. On wort gelatine and glucose-peptone gelatine the growth is tufted, the gelatine being darkened and liquefied.

Chlamydospores are formed on all media and irregular swellings occur in the hyphae on glucose-peptone agar.

Zygospores are formed on all media, but are not numerous on Czapek's medium. On glucose-peptone agar the zygospores are yellower than the hyphae, and can be seen with the naked eye. They

range up to 0·5 mm. in diameter when the hyphae have invested the zygote (Fig. 7*f*).

Zygospores develop in the fluffy aerial mycelium and examination is difficult, because of the abundant production of oil. On corn-meal agar the zygospores appear to become more mature. There are fewer surrounding hyphae and much less oil in connection with the zygote. The large zygospore can be seen through the hyphal investment, and when mature it has a thick brown roughened coat (Fig. 7 *g*, *h* and *i*). A definite clear wall which is thought to be formed from the compacted hyphae encloses the spore. The diameter of the whole zygospore, including the coat of hyphae, is 50–60μ, that of the spore itself 30μ. The hyphae which give rise to the zygospore, can often be seen as two fine strands arising from the same branch of the mycelium (Fig. 7 *g*). The observations were made in culture about eight weeks old.

As the sporangiophore is unbranched, this species was compared with those grouped together, by Dauphin as simple forms. Of these, *M. Rostafinski* Bref. is described as bearing zygospores, but it differs in having a rhizoidal base and a constriction below the sporangium. Its spores are larger and the species will grow on horse manure. *M. renispora* could not be cultured on this medium.

Of the forms described by authors subsequent to Dauphin *M. alpina* Pey. (9), resembles *M. renispora* in its mode of growth except for the basal foot. The measurements are larger and no zygospore has been described.

The description of *M. elasson* Sid. and Paxt. shows certain resemblances, but when *M. renispora* was grown on corn-meal agar for comparison its spores were less readily produced, they were still smaller and abundant zygospores were formed.

5. *Mortierella stylospora* nov.sp. (Text-fig. 8.)

Mycelial growth always white and very fine. Chlamydospores few, terminal. Stylospores very well developed on Czapek's medium, borne on fine aerial upright hyphae, 40μ in height, thick walled and reticulated, 18μ diam. Zygospore-like clumps observed on other media.

Mycelio semper albido, pertenui, chlamydosporas paucas, terminales praebente. Stylosporis in "Czapek's medium" optime evolutis, hypha erecta tenui, 40μ alta suffultis, pachydermis, reticulatis, 18μ diam. Concretiones, quasi zygosporae sint, in "glucose-peptone agar" observatae sunt.

This species was isolated from garden soil in 1928.

On Czapek's medium the mycelium is very sparse, but on other media a thick, fluffy colony is formed; gelatine is liquefied and darkened (Fig. 8*d*).

No sporangia have been seen. The method of reproduction in

culture appears to be by means of well-developed stylospores. The reticulated outer wall of the stylospores is very pronounced on Czapek's medium, but on other media is only faintly seen (Fig. 8 *a*, *b* and *c*).

Clumps of hyphae resembling the zygospores of *M. renispora* were seen on glucose-peptone agar, but true zygospores were not observed (Fig. 8 *e*).

It seems that this form might be of interest, on account of the affinities noted by Thaxter (16) between *Mortierella* and *Endogone*.

The single-spored sporangia of *Haplosporangium decipiens* Thaxt. (15) are also comparable, but Thaxter has described a tapering sporangiophore. The presence of reticulated stylospores and suggestions of zygospore formation, coupled with the absence of affinities with other Phycomycete genera, indicate that this form probably belongs to the genus *Mortierella*.

Acknowledgments

Thanks are due to Prof. Ewart of the Melbourne Botanical School, for making available the facilities of the Department, and to Dr Ethel McLennan for her help and guidance throughout. I am indebted to Mr H. W. Allen for help with the Latin diagnoses.

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THE EFFECT OF CULTURAL CONDITIONS ON THE GROWTH AND SPORULATION OF AN ORGANISM BELONGING TO THE GROUP SPECIES *ASPERGILLUS GLAUCUS*

By B. L. CHONA, B.Sc., Ph.D.

Introductory

The experiments described were carried out with an organism belonging to the group species *Aspergillus glaucus*. Two cultures containing abundant perithecia were obtained from Mr La Touche of the Medical Department of St Mary's Hospital. These were slightly different strains, one always forming perithecia more rapidly and abundantly but growing less vigorously in a vegetative manner than the other. As very little information could be found about the conditions which control perithecial formation in cultures of *Aspergillus*, and as the fungus appeared to be suitable for work along such lines, the present investigation was undertaken.

Historical

De Bary (1), working with a form of *Aspergillus glaucus*, found that perithecial formation is stimulated by an abundance of assimilable carbohydrate. On media rich in sugar, the cultures appeared yellow because of the numerous perithecia, whereas the same organism, grown on leather or on a medium lacking sugar but containing peptone, appeared green by reason of the abundance of conidia. Fraser and Chambers (2), in their study of *A. herbariorum* Wiggers (*Eurotium Aspergillus glaucus* de Bary), found that a suitable medium for perithecium formation was a prune decoction strengthened with a high concentration of cane sugar. On this medium the fungus fruited readily about five days after the temperature had been raised to 20° C.

The importance of the temperature factor is not clearly stated by Fraser and Chambers. It appears sometimes to exert a marked effect on the kind of fructification produced. Thus Thom and Church (3) state that, at temperatures between 22° and 30° C., two strains of *A. Fischeri* produce perithecia almost to the exclusion of conidia, whereas both at high and low temperatures the cultures are green because of the abundance of conidia.

Experimental method

Growth was studied in Petri-dish cultures. Care was taken that the dishes were of the same size and that the same volume of medium was added to each. The standard plate consisted of 50 c.c. of medium in a Petri dish of 12 cm. diameter. In one series of experiments the quantity of medium was varied, as will be described later.

The following media were used, either as such or in various modifications:

(1) Prune-sugar agar.
10 medium-sized prunes*.
Cane sugar, 40 gm.
Agar, 5 gm.
Water (to make up), 100 c.c.

(2) Richards's solution agar.

(3) Czapek's solution agar (Thom and Church's modification).

(4) Brown's solution agar.

All the media were sterilised in the autoclave for twenty minutes at 15 lb. pressure. Richards's solution agar, which has a distinctly acid reaction (*p*H 4·5–5), was autoclaved in two parts, one containing the acid phosphate only, and the other the remaining constituents including the agar. After autoclaving, the two were mixed. Prepared in this way, the medium sets strongly, even with only 1·5 per cent. agar.

Plates were inoculated at the centre with conidia, after which they were, as a rule, wrapped in tissue paper to guard against contamination and incubated at 25° C.

Throughout the work both strains of the organism were used. To ensure purity, each was begun from a single hyphal tip. The behaviour of the two strains was essentially similar throughout, the quantitative difference already indicated being shown under all environmental conditions. The data given below refer to the strain which produced perithecia abundantly.

Effect of medium

After fourteen days' growth at 25° C., the following observations were made:

On prune-sugar agar: growth luxuriant, spreading with abundant aerial mycelium. A dense layer of perithecia was found on the surface of the medium, and also scattered throughout the aerial mycelium. Scattered throughout the latter and especially prominent towards the upper regions were abundant conidiophores with conidia.

On Richards's solution agar: growth moderate and less widely

* Boiled in water until they have broken down, after which the debris is strained off, the clear liquid being incorporated in the medium.

spread: no loose aerial mycelium as in the preceding, but surface of culture fairly closely set with short erect conidiophores. Perithecia in fair number, but much less prominent than on the prune-sugar agar.

On Czapek's and Brown's agars: as on Richards's agar but with reduced superficial growth, fewer perithecia and conidia.

The data are put in roughly quantitative form in Table I.

Table I

Medium	Diameter of colony (average of six plates) (cm.)	Perithecia	Conidia
Prune-sugar agar	12·0	++++++	+++++
Richards's agar	3·8	++	+++
Czapek's agar	1·8	+	++
Brown's agar	1·0	Very few	+

In the above table the media are arranged in order of diminishing concentration. It appears that concentration of nutrient runs parallel to rate of growth and intensity of formation both of perithecia and conidia. Certain qualitative differences in the media must however be of importance, as further experiments with different strengths of the same medium showed that over a considerable range of concentrations there is a negative correlation between perithecial and conidial formation.

Experiments on the effect of concentration of medium were carried out using Czapek's solution at different strengths. If the ordinary concentration be denoted by N, a range of concentrations from $16N$ to $N/16$ was tested. The experiment was carried out in 6-plicate, and after three weeks' growth gave the results shown in Table II.

Table II

Concentration	Average diameter (cm.)	Perithecia	Conidia
$16N$	7	++++	+
$8N$	8	++++	+
$4N$	6	++++	+
$2N$	5	++	++
N	4	+	+++
$N/2$	3·5	+	+++
$N/4$	2·5	+	++++
$N/8$	2·5	+	+++
$N/16$	2·0	+	+++

A similar series of experiments was carried out with various strengths of Richards's solution (N ranging to $N/8$). The results were quite comparable to those shown in Table II.

At the higher concentrations the cultures are yellow, because of perithecial formation, whereas conidial formation, though not suppressed, is comparatively slight. The converse is true at the lower concentrations, the cultures then appearing green.

Over the whole range of concentrations the growth resembles that on Czapek's medium. Though the highest concentration given in Table II is comparable to that of prune-sugar agar, the type of growth shown on the latter—that is, profuse development of aerial mycelium, accompanied by large conidial development—does not occur. This indicates that the type of growth shown on prune-sugar agar is not the result of high concentration only, but that qualitative factors such as perhaps the nature of the nitrogenous constituent are also of importance.

The negative correlation between perithecial and conidial formation was also illustrated in an experiment in which the quantity of medium was varied. Plates of standard size were set up with different quantities of Czapek's medium. After sixteen days' growth the relative abundance of perithecia and conidia with different amounts of medium were as shown in Table III. There was no significant difference in size of the various colonies.

Table III

Quantity of medium (c.c.)	Perithecia	Conidia
50	++++	++
30	++++	++
20	+++	+++
10	++	+++

Experiments were also carried out in which the concentration of individual components of the cultural medium (synthetic) was varied or in which certain of the components were replaced by compounds of similar chemical nature. The medium chiefly employed was Czapek's solution.

The three constituents—magnesium sulphate, potassium chloride and ferrous sulphate—are of no importance. When these are omitted* the type of growth is quite unaltered. The remaining constituents are of greater importance, as is shown in the following account.

Potassium phosphate (K_3PO_4). When this is omitted, the amount of growth is definitely reduced. Perithecial formation, as compared with that on the complete medium, is delayed by some days and is less abundant. When the tribasic phosphate is replaced by either of the acid phosphates in equivalent amounts both growth and perithe-

* Potassium chloride is obviously unnecessary, as the needs of the fungus for potassium are supplied by the potassium phosphate. The other two constituents are considered to be essential, but it is clear that the fungus obtained all its requirements of the elements Mg, S and Fe from impurities, *e.g.* from the agar.

cial formation are slightly increased. The effect is more pronounced with the di-acid salt.

Sodium nitrate. When this is omitted, the cultures are extremely thin, though the rate of superficial growth is much the same as on the complete medium. The formation both of perithecia and of conidia is extremely scanty. Perithecia are late in appearing.

When sodium nitrate is replaced by ammonium chloride, growth is not so good. Conidia are somewhat more abundant while perithecia are considerably reduced in number. The culture is of a more intense green than are cultures on the ordinary Czapek's medium.

When the combination $NaNO_3 + K_3PO_4$ is replaced by $NH_4Cl + KH_2PO_4$, a very abnormal appearance is shown. Vegetative growth is very poor, and there are very few perithecia or conidia. The whole colony is of a peculiar yellow and microscopic examination shows that the hyphae have a much enlarged beaded appearance. Both the constituents, ammonium chloride and acid phosphate, have obviously combined to give an over-acid reaction in the medium.

Sugar. Variation of this constituent produces the greatest effects. As the concentration of sugar is reduced, growth is cut down, both in quantity and in superficial area. Formation, both of conidia and of perithecia is reduced, of the latter especially. When the sugar constituent is omitted, growth is very feeble with meagre conidial formation, and with only an occasional perithecium appearing after several weeks.

When the concentration of sugar is increased beyond the normal amount (3 per cent. for Czapek's medium), growth and perithecial formation are increased but the crop of conidia is reduced.

Precisely similar results were obtained in a series of tests with modifications of Richards's solution.

In one experiment the sugar of Richards's solution was replaced by the same concentration of peptone. The cultures arising on these plates showed a markedly fluffy or mycelial type of growth. Formation of conidia was much intensified, and there was a corresponding reduction in the number of perithecia. This finding was of interest as it suggested an explanation of the different kind of growth shown on prune-sugar agar as compared with that on equally rich modifications of Czapek's solution agar. The markedly mycelial type of growth shown on the former medium seems to be due, not to the high sugar content, but to protein constituents.

The effect of acidity or alkalinity was tested with Czapek's solution. Over the range examined, viz. from 0·16 per cent. alkaline (sodium bicarbonate) to 0·16 per cent. acid (as malic acid), the effects observed were slight. Perithecial formation was somewhat less on the alkaline side. Similarly, conidia were at first somewhat less

abundant in the acid media, but this difference more or less disappeared as the cultures became older.

Effect of temperature. This was tested with two media, Richards's solution agar and prune-sugar agar. The temperature range was from 12° to 40° C.

The optimum and maximum temperatures for growth are distinctly lower on Richards's solution than on the prune medium, as the following figures show:

R.S. Opt. 25° C.; Max. between 30° and 35° C.

Prune. Opt. near 30° C.; Max. near 40° C.

On both media there is a clear positive correlation between amount of growth and abundance of perithecia and conidia. At the optimum temperature for growth, both types of fruiting structure appear soonest and in greatest quantity, and the farther the temperature is removed from the optimum point, either above or below, the slower in developing and the fewer in number are both perithecia and conidia. The behaviour of this fungus in relation to temperature is thus different from that recorded by Thom and Church (*loc. cit.*) for *A. Fischeri.*

Effect of humidity. Plates were exposed to atmospheres of different water vapour content by leaving them in vessels containing different concentrations of sulphuric acid. The various acid solutions were placed in the bottoms of large "potato" dishes and the Petri dishes were sealed to the inner surface of the lids with a solution of gelatine to which a little mercuric chloride had been added. When the gelatine had set, the cultures were exposed upside down inside the larger dish. The solutions used, which gave a graded series of atmospheric humidities, were water, 0, 10, 20, 30 and 40 per cent. sulphuric acid. As cultural media both Richards's and Czapek's solutions were used.

The optimal condition for growth was found in the vessels containing 10 and 20 per cent. sulphuric acid. Above and below these concentrations, the amount of growth as measured by superficial spread or by visible amount fell off. At the optimal humidity there was noticeably a greater development of aerial mycelium than in the plates maintained over water or in control plates kept in the usual way.

No differential effect on perithecial and conidial formation was seen, *i.e.* the abundance of both types of fructification ran parallel to the general vigour of growth of the culture.

Effect of light. This was tested in cultures on Richards's, Czapek's and prune agars. The effect was similar in all cases.

Exposure to strong light, as for example close to a window in summer time, retards growth and strongly reduces perithecial formation. Conidia are freely formed in the light. Conversely in

darkness or in a poor light, superficial growth is greater and there is strong formation of perithecia with partial suppression of conidia. The effect is clearly seen by maintaining cultures for some time in the dark and then transferring to a window ledge or vice versa. The following description illustrates the appearances seen:

(1) Cultures maintained in darkness throughout—strong yellow appearance due to abundant perithecia: conidia also present but few.

(2) Cultures maintained in darkness for one week then transferred to light. These are similar to (1) when transferred, but the new growth is green, with strong conidial formation and very few perithecia. The central yellow region later becomes greener through the formation of conidia.

(3) Cultures kept in light for one week, then transferred to darkness. Growth for the first week is obviously slower and sparser and is green from abundant conidia. After a week in darkness, the new growth is seen to be yellow and to be densely covered with perithecia.

(4) Cultures kept in light throughout—similar to the condition of (3) after one week.

The fluffy growth characteristic of cultures grown on prune-sugar agar in darkness or in subdued light is suppressed when cultures are grown in a strong light.

Summary

The two factors which chiefly influence the growth-form of *Aspergillus glaucus* are the concentration of the medium, more particularly as regards sugar, and the degree of illumination. These factors operate as follows:

1. Under starved conditions, amount of growth and sporulation (both conidial and perithecial) are small. With increase of concentration there is a parallel increase in amount of growth and sporulation of both kinds, but this only continues up to a point. Beyond a certain concentration, conidial formation decreases, whereas growth and formation of perithecia continue to increase.

2. Strong illumination represses vegetative growth and perithecial formation but stimulates conidial formation; darkness has the reverse effect.

APPENDIX

Repeated attempts were made to germinate the ascospores from perithecia of the two strains studied in this work, but without success. These tests were carried out with ascospores from cultures on different media and from cultures grown at different temperatures; with ascospores from perithecia varying from one week to eight months old; with ascospores from cultures which were completely dried off in a desiccator over calcium chloride; with ascospores which were subjected to the digestive action of acid pepsin for various periods and at various temperatures; and with ascospores from perithecia which had been heated for a short time at various temperatures. In no case has the germination of a single ascospore been seen.

This difficulty does not seem to have been experienced by other workers. With the strain of *Aspergillus glaucus* used by Fraser and Chambers, the ascospores seem to have germinated quite freely. That strain furthermore differs in the labile nature of the perithecia. Fraser and Chambers state that with their strain the perithecial wall soon degenerates, thus liberating the ascospores. With both strains dealt with in this work, the perithecial wall persists throughout, *e.g.* in cultures which are now eleven months old, the perithecial walls are still intact.

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THE OCCURRENCE IN ENGLAND OF A POTATO WILT DISEASE DUE TO *FUSARIUM OXYSPORUM* SCHLECHT.

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(With Plate XIV and 1 Text-figure)

Introductory

The diseased material was obtained late in the growing season of 1928, from an experimental plot of potatoes (Kerr's Pink variety) growing at the South-Eastern Agricultural College, Wye, Kent. Affected plants, which were fairly numerous, showed a wilted appearance in the haulm, similar to that shown in Black-leg disease, *i.e.* the leaves were inrolled and soft. When pulled up, the plants did not show the black base characteristic of the disease due to *Bacillus phytophthorus*.

Examination of the diseased material

In order to isolate the causal organism, isolations from near the base of the diseased stems of four plants were made by the usual pathological technique. Out of fifty attempts at isolations, a fungus was obtained in thirty-five; in the remainder there was no growth. Twenty-nine of these isolations gave rise to cultures of a *Fusarium*. These were shown to be the same organism by detailed examination of their growth and appearance on a variety of media.

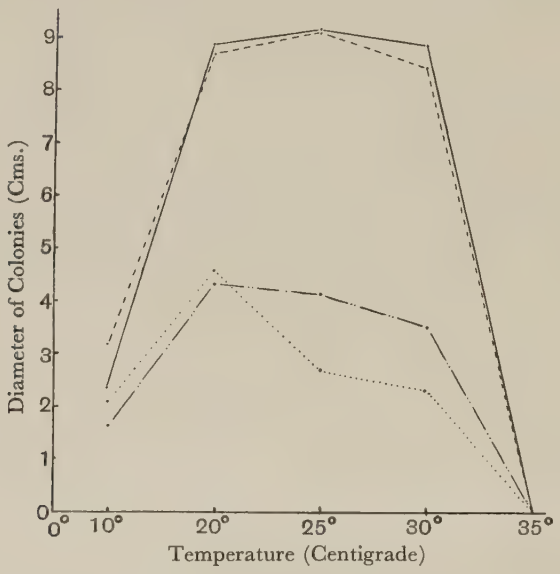
The remaining six isolations gave the following organisms: *Alternaria* sp., *Fusarium* sp. (different from the preceding), *Colletotrichum* sp. and three unidentified sterile fungi.

As the first-mentioned *Fusarium* sp. occurred most frequently it was selected for further study. No experiments were carried out with any of the other fungi isolated. A hyphal tip culture (2) from one of the twenty-nine isolations of *Fusarium* sp. was made to ensure its purity. For convenience this fungus will be referred to as *Fusarium X*.

Cultural characters of *Fusarium X*

The fungus was grown on potato extract agar, Brown's agar and Richards's agar at 10°, 15°, 20°, 25°, 30° and 35° C. At the same time *Fusarium oxysporum* Schlecht., *Fusarium eumartii* Carp., both obtained from Holland, and *Fusarium caeruleum* (Lib.) Sacc. (stock culture of Imperial College) were grown under identical conditions for comparison.

Fusarium X closely resembles *F. oxysporum*. It produced a white cottony type of mycelium at all the temperatures and on all the media tested, thus differing from *F. eumartii* and *F. caeruleum*, but agreeing with *F. oxysporum* in the following particulars: (i) occurrence of chlamydospores in the mycelium and spores of old cultures; (ii) abundance of microspores; (iii) distribution of macrospores thinly and more or less singly over the mycelium; (iv) similar growth-rate at different temperatures and on a variety of media. Text-fig. 1, which gives the growth after eight days on Brown's medium, illustrates this point.



Text-fig. 1., illustrating growth of certain *Fusarium* cultures on Brown's agar.

F. oxysporum (Holland) ——— *Fusarium X* ------
F. eumartii —••—•— *F. caeruleum* .....

It will be seen that *Fusarium X* and *F. oxysporum* agree very closely in rate of growth and in the position of the optimum. Similar curves which were constructed for growth on Richards's agar and Potato-extract agar showed exactly the same relationships.

Fusarium X agrees closely with *F. oxysporum* as regards the dimensions and septation of its spores. Figures for degree of septation in terms of 100 spores of each fungus are given in Table I. The data refer to cultures at 25° C. on Brown's agar.

Under the same cultural conditions the range of spore length was as follows:

F. eumartii	5–20 μ	Average length	= 9·7 μ
F. caeruleum	10–35 μ	,,	= 26·5 μ
F. oxysporum	5–25 μ	,,	= 12·6 μ
Fusarium X	5–35 μ	,,	= 14·7 μ

A typical sample of spores of *Fusarium X* is shown in Pl. XIV, fig. 1. Some mycelial and sporal chlamydospores are also shown in the same figure.

All these results showed that *Fusarium X* was very similar to the named culture of *F. oxysporum*. Comparisons made at other temperatures and on other media pointed to the same conclusion. A sample culture was sent to Dr Wollenweber, who identified it as *F. euoxysporum* Wr. (= *F. oxysporum* Schlecht.).

Table I

	Degree of septation				
	0	1	2	3	4
F. eumartii	86	14	0	0	0
F. caeruleum	15	26	5	54	0
F. oxysporum	51	34	15	0	0
Fusarium X	60	26	4	8	2

Pathogenicity of the fungus

This was tested both on growing plants and tubers.

The method of inoculating growing plants was to make a fairly deep longitudinal cut in the stems just above the soil level. A portion of mycelium (with spores) was inserted into the wound, the edges of which were then pressed together, wrapped round with moist cotton-wool and finally covered with tin-foil. The cotton-wool covering was moistened from time to time during the three days following inoculation. Control plants were treated in a similar way except that the inoculum was omitted. Cultures of all the four organisms mentioned above were used in these experiments.

In the first experiment tubers of Kerr's Pink were planted in pots in a cold frame at the Chelsea Physic Garden. When the shoots were about one foot high, inoculations were made. Altogether twelve shoots were inoculated with each of the four fungi. These plants together with the uninoculated controls were kept in a cold frame. No disease had developed in any after two months, by which time all the plants were pot bound.

In a second experiment, carried out at the same place and again with plants growing in pots, the experimental plants were divided into two lots, one of these being kept in a cool frame and the other in a warmed green-house. Again no infection was observed in the plants kept in the cool frame, but some attack resulted at the higher temperature (22–25° C.). *Fusarium X*, *F. oxysporum* and *F. eumartii* produced wilting symptoms in about 10 per cent. of the inoculated shoots. The inoculations with *F. caeruleum* on the other hand gave negative results. From the wilted shoots re-isolations were made and

no difficulty was experienced in obtaining again from each the organism with which it had been inoculated.

The experiments just described were carried out early in the growing season (February–June) and under the conditions prevailing the control plants were not normal. They definitely suffered from limitation of root space and the shoots were somewhat drawn up.

More convincing results were obtained when plants of Kerr's Pink growing in the open at Chelsea Physic Garden and at the Biological Field Station, Slough, were inoculated in the manner already described in the second week of June. No symptoms of disease appeared till the middle of August (after a spell of hot dry weather), when typical wilting appeared both at Chelsea and at Slough. The data of infections observed at the two places are given in Tables II and III.

Table II (*Chelsea Physic Garden*)

Fungus	No. of plants	No. of shoots inoculated	No. of shoots infected
F. eumartii	4	13	7
F. oxysporum (Holland)	5	28	13
Fusarium X	11	43	20
F. caeruleum	5	7	0
Controls	4	20*	0

Table III (*Slough*)

Fungus	No. of plants	No. of shoots inoculated	No. of shoots infected
F. eumartii	9	25	17
F. oxysporum (Holland)	7	25	22
Fusarium X	19	49	29
F. caeruleum	6	22	0
Controls	3	15*	0

* Wounded but not inoculated.

It will be seen that no infection was obtained with *F. caeruleum* at either station. This is in accordance with the findings of other workers, *e.g.* Pethybridge(7) and Wollenweber(8), that *F. caeruleum* causes a tuber rot only. The infected plants showed the rolling, yellowing and wilting of the foliage such as were described for the original diseased plants from Wye. Pl. XIV, figs. 2 and 3, illustrate the contrast between healthy and diseased plants growing at Slough.

Portions of the diseased shoots several inches above and below the points of inoculation were taken, sterilised on the surface in the usual way and placed on nutrient agar. About twenty such pieces were taken from plants inoculated with each of the three active fungi and in each the particular organism grew out and was readily isolated and identified.

Hand sections of the diseased stems both above and below the

point of inoculation showed the presence of fungal hyphae in the wood parenchyma and vessels of the vascular tissue (Pl. XIV, fig. 4).

Inoculations of potato tubers

Healthy tubers (Kerr's Pink) were inoculated by the plug method of Granger and Horne(4). The surface having been washed with a solution of mercuric chloride and carbolic acid, two plugs were cut out of each potato. One of these was inoculated, the other served as control. The experimental tubers were then placed in damp containers at laboratory temperature. After four weeks the average weight of rotted tissue (ten potatoes) produced by *Fusarium X* was 4·35 gm. The control wounds meanwhile remained perfectly sound. Again no difficulty was experienced in re-isolating the fungus from the diseased tissue.

The other three fungi, *F. oxysporum*, *F. eumartii* and *F. caeruleum*, were similarly tested, and were likewise shown to be capable of producing tuber rot.

The degree of humidity of the atmosphere in which the potatoes are maintained appears to exert considerable effect on the amount of tuber rot produced. This is illustrated in Table IV, which gives the average amounts of rot produced on twelve tubers under different conditions of storage. One set of tubers was stored in containers which were artificially kept moist by means of wet filter papers. The other set was placed in dry dishes.

Table IV

		Av. amount of rot produced	
		Fusarium X	*F. caeruleum*
Temp. °C.	Atmosphere	gm.	gm.
20	Dry	0·46	4·3
	Moist	4·55	20·6
25	Dry	0·71	0·07
	Moist	1·0	0·5
30	Dry	trace	0
	Moist	trace	0

It will be seen that with both fungi, moist atmospheric conditions increase the amount of attack. At 20° C. *F. caeruleum* is a more vigorously rotting organism than *Fusarium X*, but the attack of the former falls off much more sharply as the temperature of storage is raised. The latter result is in accordance with the previous finding that *Fusarium X* has a higher optimum for growth than has *F. caeruleum*.

In a series of standardised inoculations carried out with *F. caeruleum* it was found that the amount of attack increases with advancing age of the tubers. Thus the amount of rot produced in four weeks (December 4th–January 3rd), the temperature being maintained at

20° C., was fully twice that obtained in a similar test lasting from November 1st to 30th. This result is in agreement with general experience of this storage rotting organism. Nevertheless when greened sprouted tubers were inoculated with *F. caeruleum* in the following January, no attack of the sprouts and very little attack of the tubers resulted. This result suggests that the normal fall of resistance with period of storage may be reversed under certain conditions. Whether the determining factor is greening or sprouting or desiccation of the tuber was not established.

Discussion of results

The experiments described prove that the disease which occurred at Wye in 1928 was due to a wilting organism, and that this was *Fusarium oxysporum* Schlecht. This type of disease has hitherto not been reported for Great Britain, and it is not mentioned in the list of diseases of economic plants published by the Plant Pathological Sub-Committee of the British Mycological Society(1).

F. oxysporum causes a wilt disease of potato (Haskell(5) Heald(6)), widely spread in the southern United States. The organism may produce a tuber rot under certain conditions. In Europe the disease appears to be much less prevalent: according to Wollenweber(8) it does not occur in Germany or in northern Europe but has been reported from the south of France. Wollenweber considers that it is a purely vascular disease and that the organism is unable to attack tubers.

The organism isolated from diseased potatoes at Wye is certainly able to attack tubers in storage, though at ordinary temperatures its activity in this respect is much less than that of such a typical tuber-rotting fungus as *F. caeruleum*.

The late summer of 1928 was unusually hot in the south of England; that circumstance was probably responsible for the natural occurrence of the disease. The summer of 1929 was again very hot, and it is quite likely that if it had been otherwise no success would have been obtained in the field inoculations. The disease is of the high temperature kind, showing up only after a spell of hot dry weather, and not likely to be of great importance under normal English conditions. According to Goss(3) a soil temperature approximating to 30° C. and a low soil moisture-content are factors which favour the development of this disease.

My thanks are due to Prof. W. Brown, who supplied me with the original infected plants and who has advised me throughout the course of the work.

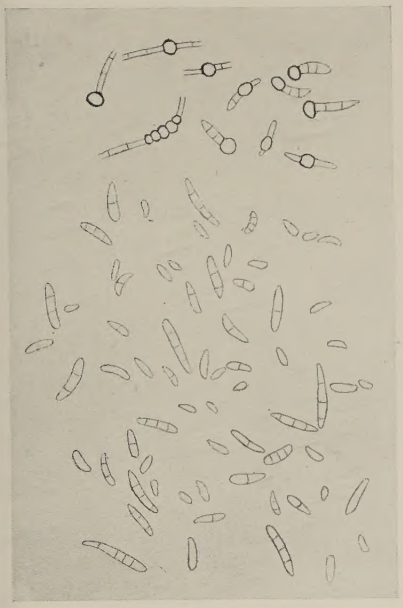


Fig. 1

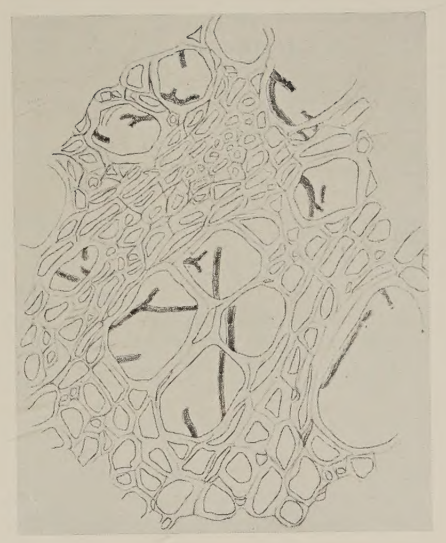


Fig. 4

Fig. 2

Fig. 3

EXPLANATION OF PLATE XIV

Fig. 1. Spores of *F. oxysporum* (the writer's isolation) with some sporal and mycelial chlamydospores at the top. (Camera lucida drawing, × 150.)

Fig. 2. (Right) one healthy control plant and (left) plants infected with *F. oxysporum* (as above).

Fig. 3. (Right) one control plant and (left) plants infected with *F. oxysporum* (Holland culture).

Fig. 4. Transverse section of infected potato stem showing hyphae of *F. oxysporum* (writer's strain) in the xylem. (Camera lucida drawing, × 160.)

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REVIEW

Hausschwamm-Merkblatt. Was jedermann, ob Hausbesitzer oder Mieter, auch der Baufachmann vom Hausschwamm und anderen holzzerstörenden Pilzen wissen muss. By F. Kallenbach. pp. 23, tt. 8. Darmstadt: Hessische Landestelle für Pilz- und Hausschwamm-Beratung. 1932. 4 Mk.

The interest in the destruction caused by *Merulius lacrymans* in buildings has increased greatly in this country since the war. So much so that there is a move to ensure that all constructive timber should be treated with some sort of preservative; I am told that no government building subsidy is given in Denmark unless the timber is treated according to a certain specification.

It is a little surprising to find that in spite of the fact that much of the fundamental work on dry-rot has been done in Germany, a popular campaign appears to have been necessary to explain the seriousness of the position. F. Kallenbach, well known as an authority on *Boletus*, has produced an interesting booklet in which he is described as "Direktor der Hessischen Landesstelle für Pilz- und Hausschwamm-Beratung." A coloured poster (six colour, 75 × 60 cm.) accompanies the pamphlet.

The first twelve pages of the pamphlet give descriptions of the eight excellent plates—one an uncoloured reduction of the poster, the others each containing four photographs. The series is excellent, showing the various stages of *Merulius* and its depredations.

The letter press is written in popular style and gives a clear description of the essential points. The remaining pages complete the general account of the fungus and methods of treatment.

The pamphlet gains additional authority by having been read through by Professor R. Falck: it is well produced and in addition to serving its immediate purpose should be found useful in other countries.

J. R.